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ÇUKUROVA UNIVERSITY
HEALTH SCIENCES INSTITUTE
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**The contribution of serotonergic and noradrenergic system to
the antidepressant-like effect of tramadol in the unpredictable
chronic mild stress model**

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**Kronik öngörülemeyen hafif stres modelinde tramadol'ün
antidepresan benzeri etkisine serotonerjik ve noradrenerjik
sistemin katkısı**

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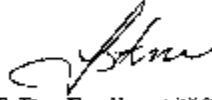
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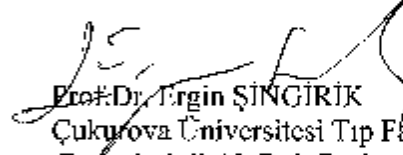
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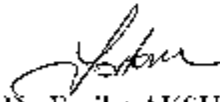
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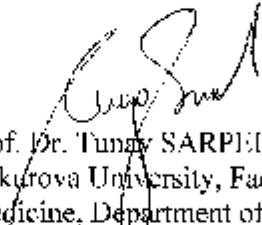
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SYMBOLS and ABBREVIATIONS

α -MPT	α -methyl-para-tyrosine
5, 7-DHT	5, 7-dihydroxytryptamine
5-HT	5-hydroxytryptamine, serotonin
5-HTP	5-hydroxytryptophan
5-HIAA	5-hydroxyindolacetic acid
ACTH	Adrenocorticotrophic Hormone
AP	Anteriposterior
APA	American Psychiatric Association
Ca ⁺²	Calcium
CCK	Cholecystokinin
CNS	Central Nervous System
COMT	Catechol-O-methyl-transferase
CRF	Corticotropin releasing factor
CRH	Corticotropin releasing hormone
CSF	Cerebrospinal fluid
DA	Dopamine
DOPA	Dihydroxyphenylalanine
DOPAC	3,4 dihydroxyphenylacetic acid
DRN	Dorsal Raphe Nucleus
DSM IV	Diagnostic and Statistical Manual of Mental Disorders
ECT	Electroconvulsive therapy
FST	Forced Swimming Test
GABA	γ -aminobutyric acid
HVA	Homovallinic acid
HPA	Hypothalamic pituitary adrenal
HPLC-ED	High Pressure Liquid Chromotography-Electrochemical detection

HPT	Hypothalamo pituitary thyroid (HPT)
ICD-10	International Classification of Disease
I.C.V	Intracerebroventricular
I.P	Intraperitoneal
K ⁺	Potassium
KO	Knockout
Lat	Lateral
LC	Locus coeruleus
LSD	Lysergic acid diethylamide
LT	L-tyrpyophan
MAOIs	Monoamine Oxidase Inhibitors
MHPG	3-methoxy-4 hydroxy-phenylglycol
MRN	Median Raphe Nucleus
NA	Noradrenaline
NARIs	Noradrenaline reuptake inhibitors
NaSSA	Noradrenergic and selective serotonergic antidepressant
NAT	Noradrenaline transporter
NMDA	N-methyl-D-aspartate
PCPA	Para-chlorophenylalanine methyl ester
PET	Positron Emission Tomography
SERT	5-HT transporter, Serotonin transporter
SNRIs	Serotonin Noradrenaline Reuptake Inhibitors
SSRIs	Selective Serotonin Reuptake Inhibitors
REM	Rapid Eye Movement
TCA	Tricyclic Antidepressants
TMS	Transcranial Magnetic Stimulation
TST	Tail Suspension Test
UCMS	Unpredictable Chronic Mild Stress
V	Vertical
VIP	Vasoactive intestinal peptide
VNS	Vagus Nerve Stimulation
WHO	World Health Organisation

ABSTRACT

The Contribution of Serotonergic and Noradrenergic System to the Antidepressant-like Effect of Tramadol in the Unpredictable Chronic Mild Stress Model

Tramadol is a centrally acting clinically effective analgesic which inhibits noradrenaline (NA) and serotonin (5-HT) reuptake. This study was planned to investigate the possible antidepressant-like effects of tramadol (20 mg/kg) in the unpredictable chronic mild stress (UCMS) model of depression in BALB/c mice and we also searched the participation of serotonergic and noradrenergic system. Desipramine was used like a positive control. Tramadol and desipramine reversed the physical and behavioural abnormalities induced by the UCMS. Furthermore, the lesion of the dorsal raphe nucleus (DRN) by 5, 7-dihydroxytryptamine (5,7-DHT) antagonized the antidepressant-like effects of tramadol and desipramine on the coat state, in the splash test in stressed mice but not non-stressed mice.

The results obtained by High Pressure Liquid Chromatography (HPLC) showed that level of the 5-HT and its metabolite 5-hydroxyindolacetic acid (5-HIAA) was reduced by the 5,7- DHT lesion in some brain regions. In contrast, the level of NA, Dopamine (DA), Homovanillic acid (HVA) and 3, 4 dihydroxyphenylacetic acid (DOPAC) did not change by the 5, 7-DHT lesion. Moreover the UCMS regimen diminished the level of 5-HT, 5-HIAA, NA and DA in some brain regions.

Furthermore, the contribution of the 5-HT_{1A} receptors to the antidepressant-like effects of tramadol was searched by the 5-HT_{1A/1B} receptor antagonist pindolol (10 mg/kg); we observed neither significant acceleration nor diminution by pindolol on the actions of desipramine and tramadol.

In the last part, we measured the NA and its metabolite 3-methoxy-4-hydroxy-phenylglycol (MHPG) levels via HPLC in stressed mice. The chronic treatment with desipramine and/or tramadol significantly augmented MHPG and/or NA level in the locus coeruleus (LC), hypothalamus and hippocampus but not in cerebellum. Moreover, desipramine and tramadol induced antidepressant-like effects in the UCMS model can be blocked by propranolol (non-selective β -adrenoreceptor antagonist, 5 mg/kg), ICI 118,551 (selective β_2 -adrenoreceptor antagonist, 2 mg/kg) and yohimbine (selective α_2 -adrenoreceptor antagonist, 2 mg/kg).

Taken together these results suggest that tramadol has an antidepressant-like effect in the UCMS model, which is mediated by both serotonergic and noradrenergic system.

Key Words: Unpredictable chronic mild stress, tramadol, serotonergic system, noradrenergic system, mice

RESUME

Implication des Systèmes Sérotonergique et Noradrénergique dans les Effets du Tramadol dans le Modèle du Stress Chronique Léger Imprédictible.

Le tramadol est un analgésique cliniquement efficace qui inhibe la recapture de la noradrénaline (NA) et de la sérotonine (5-HT).

Cette étude a été planifiée dans le but d'étudier les effets anti-dépresseurs du tramadol (20mg/kg) dans un modèle de dépression : le stress chronique léger imprédictible (SCLI), chez les souris BALB/c. Nous avons également étudié l'implication des systèmes sérotonergique et noradrénergique dans les effets du tramadol dans ce modèle de SCLI. La désipramine a été utilisée comme contrôle positif lors de cette expérience. Le tramadol et la desipramine ont inversé les effets physiques et comportementaux causés par le SCLI. De plus, la lésion du noyau du Raphe dorsal (Dorsal Raphé Nucleus : DRN) avec de la 5,7 dihydroxytryptamine (5,7- DHT) a antagonisé les effets anti-dépresseurs du tramadol et de la desipramine chez les souris stressés mais non chez les souris non stressés. Les résultats obtenus avec la technique de l'HPLC (High Pressure Liquid Chromatography) ont montré que le niveau de 5-HT et de son métabolite, la 5-hydroxyindolactétique acide (5-HIAA) ont été réduit dans certaines parties du cerveau chez les souris portant des lésions à la 5,7 DHT. A l'inverse, le niveau de NA, de Dopamine (DA), d'acide Homovanillique (HVA) et d'acide 3,4 dihydroxyphenylacétique (DOPAC) n'ont pas changés chez les souris porteuses de lésions à la 5,7 DHT. De plus, dans le groupe SCLI, le niveau de 5-HT, de 5-HIAA, de NA et de DA diminue dans certaines parties du cerveau.

Nous avons également cherché la contribution des récepteurs 5-HT_{1A} dans les effets anti-dépresseurs du tramadol en utilisant un antagoniste des récepteurs 5-HT_{1A/1B}, le pindolol (10mg/kg) : nous n'avons observé ni une accélération ni une diminution des effets de la desipramine et du tramadol induits par le pindolol.

Dans la dernière partie, nous avons mesurés la NA et les niveaux de son métabolite, le 3-methoxy-4-hydroxy-phenylglycol (MHPG) avec l'HPLC chez les souris stressées. Le traitement chronique avec la desipramine et/ou le tramadol fait augmenter de manière significative le niveau de MHPG et/ou le niveau de NA dans le locus ceruleus (LC), l'hypothalamus et l'hippocampe mais pas dans le cervelet. Les effets anti-dépresseurs de la désipramine et du tramadol peuvent être bloqués avec le propranolol (antagoniste non sélectif des récepteurs b-adrénergiques, 5mg/kg), l'ICI 118,551 (antagoniste b₂- adrénergique, 2mg/kg) et la yohimbine (antagoniste a₂- adrénergiques, 2mg/kg).

L'ensemble de ces résultats suggère que le tramadol ait un effet anti-dépresseur dans le modèle de SCLI, qui est médié par les systèmes sérotonergiques et noradrénergiques.

Mots clé: Stress chronique léger imprédictible, Tramadol, le System Sérotonergique, le System Noradrénergique, Souris

ÖZET

Kronik Öngörülemeyen Hafif Stres Modelinde Tramadol'ün Antidepresan Benzeri Etkisine Serotonerjik ve Noradrenerjik Sistemin Katkısı

Tramadol, serotonin (5-HT) ve noradrenalin (NA) reuptake'ini inhibe eden santral etkili bir analjeziktir. Bu çalışmanın amacı tramadol'ün (20 mg/kg, i.p) olası antidepresan-benzeri etkisini kronik öngörülemeyen hafif stres (KÖHS) modelinde araştırmak, serotonerjik ve noradrenerjik sistemin katkısını belirlemektir. Bu çalışmada noradrenalin reuptake inhibitörü desipramine (10 mg/kg) pozitif kontrol olarak kullanıldı. Tramadol ve desipramine KÖHS modelinin oluşturduğu fiziksel ve davranışsal bozuklukları geri çevirdi. Ayrıca 5, 7-dihidroksitriptamin (5, 7-DHT) ile oluşturulan dorsal raphe magnus lezyonu tramadol ve desipramin'in antidepresan-benzeri etkisini stres oluşturulan farelerde bloke etti. Yüksek Basıncılı Likid Kromatografisi (YBLK) yönteminden elde edilen sonuçlar, 5, 7-DHT lezyonunun 5-HT ve ana metaboliti 5-hidroksiindolasetik asid (5-HIAA) düzeyini bazı beyin bölgelerinde azalttığını gösterdi. Zıt olarak, NA, Dopamin (DA), Homovalinik asid (HVA) ve 3, 4 dihidroksifenilasetik asid düzeyleri 5, 7-DHT lezyonundan etkilenmedi. Ayrıca KÖHS uygulaması 5-HT, 5-HIAA, NA ve DA düzeylerini bazı beyin bölgelerinde azalttı.

5-HT_{1A} reseptörün tramadol'ün antidepresan benzeri etkisine katkısı 5-HT_{1A/1B} reseptör antagonisti pindolol (10 mg/kg) aracılığıyla araştırıldı. Pindolol tramadol'ün aktivitesini etkilemedi.

Bu çalışmanın son kısmında stres oluşturulan hayvanlarda YBLK yöntemi ile NA ve ana metaboliti 3-metoksi 4-hidroksi fenilglükol (MHPG) düzeyleri ölçüldü. Kronik desipramine ve /veya tramadol uygulaması MHPG ve/veya NA düzeylerini locus coeruleus (LC), hipotalamus ve hipokampus da artırırken, serebellumda herhangi bir etki göstermedi. Ayrıca, tramadol ve desipramin'in KÖHS modelindeki antidepresan-benzeri etkisi selektif olmayan b-adrenoreseptör antagonisti propranolol (5 mg/kg), selektif b₂ reseptör antagonisti ICI 118,551 ve selektif a₂-reseptör antagonisti yohimbine tarafından bloke edildi.

Çalışmanın sonuçları değerlendirildiğinde, tramadol'ün KÖHS modelindeki antidepresan-benzeri etkisini serotonerjik ve noradrenerjik sistem aracılığıyla gösterdiği kanısına varıldı.

Anahtar kelimeler: Kronik öngörülemeyen hafif stres modeli, Tramadol, Serotonerjik sistem, Noradrenerjik sistem, Fare

1. INTRODUCTION and OBJECTIVES

Depression is a common, debilitating life-threatening illness with a significant incidence in the population¹. Numerous antidepressant compounds are now available, presumably acting via different mechanisms. However, it is necessary to continue to search new compounds to improve in terms of latency of onset of therapeutic action, side effect profile and treatment of non-responders. It is a truism that clinically effective drugs for mood disorders such as imipramine, iproniazid had been initially used for their different actions instead of their antidepressant effects². For this reason, it seems worthwhile to search the possible antidepressant-like effects of drugs, which are currently used for their clinical purposes. For example, tramadol, a widely used 'atypical' opioid analgesic which inhibits neuronal reuptake of serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline (NA)^{3, 4}, has structural similarities to the clinical antidepressant venlafaxine⁵. Furthermore, it has already been reported by the Rojas-Corrales et al.^{6, 7} that tramadol has an antidepressant-like effects in the forced swimming test (FST) in mice and learned helplessness model in rats.

Moreover, in clinical trials, tramadol is described as an effective therapeutic agent in treating obsessive-compulsive disorders⁸ and refractory major depression⁹. In line with these evidences, other studies have shown that tramadol induces changes in the central nervous system (CNS) similar to those induced with conventional antidepressants¹⁰. But to our knowledge, there is no study for the efficacy of tramadol in chronic stress model and the mechanism of antidepressant-like effect of tramadol still remains unclear.

In the light of these data, we examined the possible antidepressant-like effects of tramadol using unpredictable chronic mild stress (UCMS), a model that is generally thought to be the most promising and valuable model to study depression in rodents, mimicking several human depressive symptoms¹¹. For this purpose, we compared the effects of tramadol with the clinically used antidepressant desipramine. In addition, the mechanisms that contribute to the antidepressant-like effects of tramadol were also evaluated.

The first part of the study was planned to find out the more sensitive strain for the UCMS model, as well as to make a pharmacological validation of this model in our laboratory. For this purpose, one outbred (Swiss) and one inbred (BALB/c) mice were selected to investigate the potential contribution of genetic vulnerability in response to an UCMS model. These strains are the most frequently used strains in psychopharmacological tests¹². In order to examine the drugs action, four antidepressants which show their effects via different mechanisms were assessed; two tricyclic antidepressants (TCAs) (imipramine, a mixed serotonergic/noradrenergic reuptake inhibitor and desipramine, a specific noradrenergic reuptake inhibitor); one tetracyclic antidepressant (maprotiline which strongly inhibits the uptake of NA, though it is notable in its lack of inhibition of serotonergic uptake) and selective serotonin reuptake inhibitors (SSRI) (fluoxetine).

The state of the coat of mice was evaluated to determine the effects of UCMS and the drugs treatment in BALB/c and Swiss mice. The UCMS model incorporates assessment of the animal's coat condition, which deteriorates in line with stress-induced deficits in grooming in an antidepressant-reversible manner. In addition, the splash test was performed to realize the grooming behaviour. This study enabled us to choose the strain, which is more sensitive to the effects of UCMS model for the rest of the study. Furthermore, UCMS model was pharmacologically validated and the drug, which was used like a positive control was chosen among the drugs tested.

The second part of the study was designed to examine the possible antidepressant-like effects of tramadol in the UCMS model in BALB/c mice. In order to determine the effects of UCMS and the drug treatments, the grooming behaviour was evaluated indirectly by the assessment of the coat state and directly by the splash test. Moreover, the effects of UCMS and the drug treatment on the learning-memory were determined using the Morris water maze. For each group, locomotor activity and alterations of body weight were also evaluated.

Furthermore, we aimed to clarify the mechanisms underlying the antidepressant-like action of tramadol. As we mentioned before, tramadol has NA and 5-HT reuptake blocking properties like common antidepressants. For this reason, in the third part of the study, we sought the participation of the serotonergic system in the effects of tramadol,

as it is one of the most studied system for mood disorders. So, in this part of the study, the effects of a 5, 7 dihydroxytryptamine (5, 7-DHT) lesion of the dorsal raphe nucleus (DRN) on the antidepressant-like effects of tramadol and desipramine were examined by the behavioural tests and the biochemical methods. The DRN is the largest serotonergic nuclei of the brainstem containing about 50% of 5-HT neurons in the rat CNS and 50-60% in human CNS¹³ which projects to limbic system. To prevent the effects of serotonergic system on the DRN, 5, 7-DHT, which is an analog of 5-HT that destroys 5-HT axons and terminals was used. To evaluate the effects of the lesion or the drug treatments, the evaluation of coat state, body weights and locomotor activity and the splash as well as the resident-intruder tests were realized. After the behavioural tests, we aimed to determine the effects of the lesion, the stress procedure and drug treatment on the monoamine level of some brain regions which are important areas for the depressive disorders and serotonergic system by the high pressure liquid chromatography (HPLC) method.

In addition, the participation of the 5-HT_{1A} receptors, which is the most extensively studied among the 5-HT receptors was examined via 5-HT_{1A/1B} receptor antagonist pindolol in the antidepressant-like effects of tramadol and desipramine. We sought whether pindolol can change the onset of the effects of tramadol and desipramine. This was done because it has been reported that pindolol can accelerate the antidepressant response to the SSRI paroxetine, presumably by preventing the initial decrease in firing activity of 5-HT neurons produced by the SSRI¹⁴.

In the last part of this study, we ascertained the contribution of the noradrenergic system to the antidepressant-like effects of desipramine and tramadol. First, we aimed to seek the effects of tramadol and desipramine on the level of NA and of its main metabolite MHPG in mice after the UCMS regimen. Measurement of MHPG in body fluids such as cerebrospinal fluid, plasma and urine has been most widely applied to the studies of depression, but there is no recent study involving brain tissues. So, we investigated the NA and MHPG concentration in different brain tissues, which are related to the noradrenergic system and have also a role in psychiatric disorder, such as the region of Locus coeruleus (LC), the hippocampus, the hypothalamus and the cerebellum.

Further, we focused on the implication of β and α_2 adrenoceptors in the antidepressant-like effects of tramadol and desipramine. Indeed, the up-regulation of β and α_2 adrenergic receptors has been consistently found after the chronic stress and their down-regulation is regarded as a marker for antidepressant activity¹⁵⁻¹⁷. For this purpose, we used a non-selective β -adrenergic receptor antagonist propranolol to determine the contribution of β -receptors and then the selective β_2 -receptor antagonist ICI 118,551 to clarify the participation of subtype of β -receptors. In addition, we tested the effects of α_2 -adrenergic receptor antagonist yohimbine on the antidepressant-like action of tramadol and desipramine. In order to determine the effects of UCMS regimen and of the drug treatment, we examined the state of the coat of mice and we made a splash test to evaluate the grooming behaviour. Actograph was also performed to measure the locomotor activity.

2. GENERAL INFORMATIONS

2.1. Depression

2.1.1. Definition

The new, intensive focus on depression as a widespread disease has been underpinned by the work of nosologists, specialists in classifying and defining illness. The foremost definitions of depression are those developed by panels of experts convened by the American Psychiatric Association (APA). The fourth edition, known as Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), was published in 1994 and is now internationally recognized as the prime definition of how to recognize depression and, implicitly, when and how to treat it. DSM-IV definitions are also closely linked to those in the World Health Organization (WHO)'s *International Classification of Diseases* (ICD-10). So, depression is a common mental disorder that is characterized by depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. Ludwig van Beethoven had explained this pathology very well in one of his letter. He wrote "...my misfortune pains me doubly, in as much as it leads to my being misjudged. For me there can be no relaxation in human society; no refined conversations, no mutual confidences. I must live quite alone and may creep into society only as often as sheer necessity demands; I must live like an outcast. If I appear in company I am overcome by a burning anxiety, a fear that I am running the risk of letting people notice my condition...such experiences almost made me despair, and I was on the point of putting an end to my life - the only thing that held me back was my art. For indeed it seemed to me impossible to leave this world before I had produced all the works that I felt the urge to compose, and thus I have dragged on this miserable existence..."¹⁸.

2.1.2. Types of Depression and Their Symptoms

As clinical and biological researchers have studied mood disorders, previously recognized clinical distinctions among patients have become appreciated and are now officially recognized in the DSM-IV. Two major mood disorders are major depressive

disorder and bipolar I disorder. Patients who are afflicted with depressive episodes alone are told to have major depressive disorder while patients with both manic and depressive episodes and patients with manic episodes alone are told to have bipolar I disorder¹⁹.

At least five of the following symptoms must be observed to diagnose of major depression: 1) depressed mood, nearly every day during most of the day, 2) marked diminished interest or pleasure in almost all activities including sex, 3) significant weight loss (when not dieting), weight gain, or a change in appetite, 4) insomnia or hypersomnia (excess sleep), 5) psychomotor agitation or psychomotor retardation, 6) fatigue or loss of energy, 7) feelings of worthlessness or inappropriate guilt, 8) impaired ability to concentrate or indecisiveness, 9) recurrent thoughts of death, recurrent suicidal²⁰. These symptoms must be present during a 2 weeks period at least and they must represent a change from a previous level of functioning.

Not all individuals show all of the symptoms, and the constellation of symptoms presented differs across individuals.

Bipolar I disorder [DSM-IV] is a type of bipolar disorder characterized by one or more manic or mixed episodes, often with a history of one or more major depressive episodes. Bipolar I disorder is characterized by tremendous up-swings and down-swings in mood, energy level, and need for sleep.

Two additional mood disorders, dysthymic and cyclothymic disorder, have also been described clinically. Dsythymia, is characterized by low mood persisting for at least 2 years or more, tends to be less severe than acute major depression, although intensity of symptoms may fluctuate. In most respects, symptoms of dysthymia and major depression are qualitatively similar, but social-motivational symptoms are more characteristic in dysthymia²¹. Moreover unlike major depression, dysthymia is not accompanied by elevated cortisol levels²².

Cyclothymic disorder is characterized by the presence of symptoms that are less severe than the symptoms of bipolar I disorder¹⁹.

The authors of DSM-IV also codified additional mood disorders including syndromes related to depression (minor depressive disorder, recurrent brief depressive disorder and premenstrual dysphoric disorder) and disorders related to bipolar I disorder (bipolar II

disorder, which is similar to bipolar I disorder, except that the individual has less severe manic episodes).

2.1.3. Epidemiology:

Forms of clinical depression, such as major depressive disorder, are common, disabling, and often chronic conditions. Depression is expected to become increasingly prevalent over the coming decades. According to projections of the WHO, by 2020 depression is predicted to be leading cause of disability, second after to cardiovascular disease²³. Currently, major depressive disorder, which is the most widely studied form of depression, has a lifetime prevalence of 10-25% for women and 5-12% for men (DSM-IV). Individuals suffering from these disorders are not only faced with their psychological well-being, but are at considerably greater risk for various somatic conditions such as heart disease and obesity²⁴.

2.1.4. Biological Factors of Depression

2.1.4.1. Monoamine Hypothesis

Monoamine neurotransmitters are small molecule neurotransmitters that contain a single amine group. Monoamines include dopamine (DA), 5-HT, NA and adrenaline. Histamine can be included in this group of neurotransmitter as well. The monoamine hypothesis of affective disorders has evolved over the past 20 years and has contributed to our understanding of the etiology of depression and the mechanism of action of antidepressants. In the early 50s, Freis²⁵ reported that high doses of antihypertensive reserpine, which produce long-lasting depletion of monoamines in the presynaptic vesicles, induces depressive-like symptoms. Another evidence supporting monoamine hypothesis is, levels of 5-HT and NA, and their metabolites, which seem to be correlated with depression. For example, patients who have low levels of a 5-HT metabolite were found to be more likely to have committed suicide^{26, 27}.

In addition, it has been reported that 5-HT_{1A}²⁸ and also 5-HT transporter promoter²⁹ polymorphism may be associated with major depression and suicide.

Since the participation of the serotonergic and noradrenergic system was reported, these signaling pathways had become the target of antidepressants. The oldest antidepressants, the monoamine oxidase inhibitors (MAOIs), increase synaptic levels of 5-HT and NA by inhibiting the enzymatic degradation of these neurotransmitters.

Furthermore, TCAs, as well as SSRIs and serotonin NA reuptake inhibitors (SNRIs), all increase synaptic levels of 5-HT and/or NA by inhibiting reuptake of the 5-HT transporter (SERT) or NA transporter (NAT), respectively³⁰.

Even if, the monoamine hypothesis is the more ancient and the more studied hypothesis to explain the pathophysiology of depression, emerging evidence indicates that the monoamine hypothesis of 5-HT and NA modulation fails to explain the whole mechanism of antidepressants. Other hypothesis, including the cytokine hypothesis of depression, the hypothalamic-pituitary-thyroid hypothesis of depression, as well as the role of brain-derived neurotrophic factor and cyclic AMP response element binding protein are also very important factors to explain the etiology of depression.

On the other hand, recent observations investigated that adult hippocampal neurogenesis is decreased by stress³¹ and increased by chronic antidepressants³² which suggest the involvement of neurogenesis in both pathogenesis and treatment of mood disorders. Indeed, Santerelli et al.³³ showed that the suppression of hippocampal neurogenesis by irradiation diminishes the effects of fluoxetine and imipramine.

2.1.4.2. Other Neurochemical Factors

The monoamine theory itself could neither explain the whole mechanism of action of antidepressants, nor could it provide a comprehensive understanding of etiology of depression. Indeed, it is well known that monoaminergic system associated with the other systems that participates the pathophysiology of depression. For example, brain 5-HT turnover have been shown to occur in association with the activation of hypothalamic-pituitary-adrenal axis (HPA) under various stressful conditions³⁴. There are numerous biological factors other than the monoamine hypothesis to help explain the pathophysiology of depression, involving a wide range of systems and mechanisms such as, neuropeptides³⁵, imidazoline receptors³⁶, neurotrophins³⁷ and phospholipids³⁸. In the area of depression research, neuropeptide systems has focused on substance P³⁹,

corticotrophin releasing factor (CRF), melanin-concentrating hormone⁴⁰ and arginine vasopressin^{35, 41}. This has led to the development of numerous compounds now in clinical trials for depression.

In addition, there are also many approaches targeting excitatory (glutamate) or inhibitory (γ -aminobutyric acid (GABA)) aminoacid systems in depression⁴². For example, chronic antidepressant administration can influence N-methyl-D-aspartate (NMDA) receptor function and receptor binding profiles as well as generate regional alterations in mRNA expression that encodes multiple NMDA receptor subunits^{43, 44}.

Furthermore, non competitive NMDA antagonists (MK-801, memantine, ketamine) that reduce glutamatergic transmission at the NMDA receptor have demonstrated antidepressant-like effects in animal models, including forced swim and tail suspension tests, inescapable stressors and in learned helplessness^{17, 42}. On the other hand, it has been reported that cerebrospinal fluid (CSF) levels of GABA may significantly decreased in depressed patients⁴⁵. It is well known that chronic administration of antidepressants or electroconvulsive therapy increase the binding and function of GABA_B receptors in the frontal cortex of mice and rats^{46, 47}. There are also considerable data supporting a relationship between GABAergic system and noradrenergic⁴⁸ or serotonergic system⁴⁹.

2.1.4.3. Neuroendocrine Regulation

The hypothalamus is centre for the regulation of neuroendocrine axis. Various neuroendocrine dysregulation have been reported in patients with mood disorders. The major neuroendocrine axis of interest in mood disorders is adrenal, thyroid and growth hormone axes.

The HPA axis mediates stress response along a chain that involves the hypothalamus, the anterior pituitary gland and the adrenal cortex⁵⁰. Signs of a hyperactive HPA axis are found in many depressed patients⁵¹ with enhanced urinary and serum cortisol levels, non suppression in the dexamethasone test⁵², increased level of circulating adrenocorticotrophic hormone (ACTH) and corticotropin releasing hormone (CRH) in CSF⁵³. Various type of antidepressants, including SSRIs, NRIs and MAOIs increase corticosteroid receptor gene expression, the capacity of brain tissue to

bind corticosteroids and steroid receptor immunoreactivity in the brain⁵⁴. In addition, it is well known that hypothalamo–pituitary thyroid (HPT)-axis is inhibited in depression⁵⁵. Thus hypothalamic peptidergic system, i.e., the HPA-axis and the HPT-axis have many interactions with aminergic systems that are also implicated in depression⁵⁶.

Stress also stimulates the release of the neuropeptide vasopressin, which is synthesized in the paraventricular and supraoptic hypothalamic nuclei. The release of vasopressin potentiates the effects of CRF on ACTH release. Vasopressin levels are reportedly increased in some patients with depression⁵⁷ and might contribute to HPA axis abnormalities observed in these individuals.

Other neuroendocrine changes that have been reported are decreased nocturnal secretion of melatonin⁵⁸, decreased prolactin release to tryptophan administration⁵⁹, decreased basal levels of follicle-stimulating hormone⁶⁰ and luteinizing hormone, and decreased testosterone levels⁶¹ in men or male mice.

2.1.4.4. Brain Areas and Depression

Major depressive disorders are considered to have a neurochemical basis in multiple signaling pathways in different brain areas, and indeed various regionally selective impairments of structural plasticity have been reported⁶². Over the last 10 years, it has also been revealed that depression may be related to neuronal networks in limbic brain structures including prefrontal cortex, cingulate cortex, entorhinal cortex, hippocampus, nucleus accumbens, ventral pallidum, amygdala and anterior hypothalamus. Moreover, it has been reported the abnormalities in many of these brain areas in depressed patients. Knowledge of the functions of these brain region gives some ideas about how they can contribute to the symptoms of depression⁶³. For example, frontal cortex and hippocampus can participate in cognitive aspects of depression such as memory impairments and feelings of worthless, hopelessness, guilt and suicidality and also regulate abnormalities in emotional behaviour. Indeed, several reports have now demonstrated that the volume of the hippocampus and frontal cortex are decreased and structural imaging studies have shown reduced gray matter volumes in depressed patients⁶⁴⁻⁶⁶. The hippocampus is one of the regions that have recently

received significant attention in mood disorders research because of neurogenesis in dental gyrus. Stress causes cell death, dendritic shrinkage, and decreased levels of neurotrophins within the hippocampus^{67, 68}, as well as a reduction in hippocampal granule cell neurogenesis³¹. Demonstration of significant atrophy of this structure in major depression, together with the recent studies showing that various types of antidepressant treatments promote the phenomenon of neurogenesis in the rat hippocampus, bring future credence to the study of the neurochemical impacts of antidepressants in this structure^{32, 33, 69}.

Notably, the amygdala, which may have a role in the neuronal circuitry involved in regulating mood, appears to be enlarged in patients exhibiting their first major episode of depression but subsequently shrinks in volume with prolonged and recurrent depression⁷⁰.

And for the hypothalamus, it has been speculated that it contributes the symptoms of depression such as too much or too little sleep, appetite, loss of interest in pleasurable activities. Indeed, at least five interacting hypothalamic peptidergic systems are currently considered to be involved in symptoms of depression, as well as three aminergic transmitter systems that innervate the hypothalamus⁵⁶.

It is well known that striatum is an important dopaminergic region involved in motivation and affect. A dysfunction of mesocorticolimbic DA system may underlie the symptoms of anhedonia, apathy and loss of interest observed in depression⁷¹. Moreover, not only by the dopaminergic neurons, but also the striatum innervates by the serotonergic fibers from the dorsal raphe nucleus (DRN)⁷².

2.1.5. Etiology of Depression

2.1.5.1. Genetic Factors

There is a growing evidence for a significant, albeit not unique, impact of genetics in both the etiology of mood disorders⁷³ and the efficacy of psychotropes⁷⁴. Family and twin/adoption studies also provide the evidence that depression has a genetic and familial background. Indeed, 1st degree relatives are 2-3 times more likely to have major depressive disorder⁷⁵.

Caspi et al.⁷⁶ were interested in finding out the answer of the question why stressful experiences lead to depression in some people but not others. And they showed a functional polymorphism in the promoter region of the SERT that moderate the influence of stressful life events on depression⁷⁶. Furthermore, there has been interest in performance differences between rodent strains on behavioural tests for antidepressants, because such differences may identify underlying constitutive factors that contribute to the vulnerability to clinical depression in humans⁷⁷. Numbers of authors have reported robust interstrain differences both in baseline performances and the response to antidepressant drugs^{12, 77, 78}.

2.1.5.2. Environmental Factors

Besides contribution of genetics, the importance of environmental influence should be noticed for the etiology of major depressive disorder. Environmental theories of the etiology of major depressive disorders emphasize the role of external events including normal life events, familial influences, school for the children, aging and child-rearing practices in triggering depression. According to this perspective, people become depressed primarily due to unfortunate circumstances that are difficult to change. In some cases, these misfortunes may include environmental disasters or personal losses; but such other factors as low socioeconomic status, oppression associated with one's sex or race, or unpleasant or frustrating relationships are also thought to contribute to depression⁷⁹.

2.1.6. Animal Models of Depression

Despite the advance made in recent years we are still limited in the nature of studies that can be performed in the clinic to examine the neuronal state of depressed patients. Preclinical studies on animals can provide crucial information on the whole range of neuronal function not accessible in humans and potentially allow us to identify novel targets for antidepressant drug development. On the other hand, there is a discrepancy between positive outcomes of candidate drugs in animal models followed

by apparent lack of efficacy in humans². For that reason, it becomes worthwhile to choose the appropriate animal models.

Numerous animal models of depression have been advanced, each having multiple attributes and limitations. At the very least, animal models must resemble the human condition in several respects, including (a) similarity in the symptom profile presented (face validity), (b) amelioration or attenuation by treatments effective in treating the human condition, and conversely not be affected by those treatments that are ineffective in attenuating the human disorder (predictive validity)⁸⁰, (c) provocation by events thought to be important in eliciting the human disorder (etiological validity), and (d) a strong theoretical rationale such as similar neurochemical processes (construct validity)⁸¹⁻⁸³. There are several examples of animal models of depression, which are based on different factors such as pharmacological agents, stress, early social isolation, genetics and neuronal deficits etc.

2.1.6.1. Models Based on Pharmacologically Induced States

2.1.6.1.1. Reserpine Model:

The 1960s was a period during which neurochemical models of depression were being proposed and refined. Reserpine model is the earliest animal model of depression. Reserpine causes a long-lasting monoamine depletion, which is a syndrome based on locomotor hypomotility and reduced body temperature. These abnormalities are antagonized by TCAs and MAOIs. However, it is not clear what is patterned with this model; acute dysphoria or true depression?

2.1.6.1.2. α -Methyl-para-Tyrosine (α -MPT) Model:

It induces catecholamine depletion via inhibition of tyrosine-hydroxylase, which produces psychomotor retardation and reduced responsiveness to reward.

2.1.6.1.3. Amphetamine Withdrawal Model:

It occurs by the amphetamine-induced down-regulation of catecholamine receptors. It also produces psychomotor retardation. In human study, it has been reported that undergoing amphetamine withdrawal induces depressed feelings.

The uses of pharmacological agents-induced depression models are limited in their face validity, these models can not mimic most of the symptoms in human depression. These tests are also acute tests where only single administration of the test compound is sufficient to produce the pharmacological effect.

2.1.6.2. Stress:

Stress is an environmental factor capable of precipitating depressive episodes in human and in animals. We know that stressful life events appear to play a major role in the etiology of depression^{76, 84-86}, thus it is plausible that an external stress may result in changes in animal behaviour, which is a model for depressive conditions. Consistently, in laboratory animals, exposure to stressful stimuli is capable to induce both behavioural and neurobiological changes which can be either reversed or prevented by antidepressant treatments⁸⁷. Therefore, many models and tests for assessing depression-related behaviour in rodents involve exposure to stressful situations.

2.1.6.2.1. Forced Swimming Test (FST):

Of these experimental procedures, FST (also known as Porsolt's test; a behavioural despair test) is probably the most widely and most frequently used model⁸⁸⁻⁹¹. The FST is based on the observation that rodents placed in an enclosed (inescapable) cylinder filled with tepid water will initially engage in vigorous escape-orientated movements, but then within minutes will exhibit increasing bouts of immobility. The validity of the test is based on the fact that a wide range of antidepressants, mainly tricyclics and MAOIs from across chemical classes, showed positive effects in maintaining swimming behaviour in the swim test⁹¹.

2.1.6.2.2. Tail Suspension Test (TST):

It is related to FST but the task is not synonymous. The mice hung upside-down by their tail, in such a situation, mice also exhibit passive immobility after minutes of futile struggling⁹². The advantage of FST and TST is providing a rapid screening assay. Despite their appeal, there are some reasonable problems for the validity of the FST and TST as models of depression. For example, the FST and TST are sensitive to acute antidepressant administration, whereas chronic treatment is required for full clinical efficacy. So they don't ensure the same long-term adaptive changes in neuronal circuitry that underlie antidepressant effects in human.

2.1.6.2.3. Learned Helplessness:

Learned helplessness is a well-established principle in psychology, a description of the effect of inescapable punishment (such as electrical shock) on animal (and by extension, human) behaviour. Learned helplessness model derives from a cognitive view of depression in which events are viewed negatively and interpreted as not controllable leading to feelings of anxiety and helplessness when dealing with them⁹³. This model originates from the observation that dogs repeatedly exposed to electric shocks that were both inescapable and uncontrollable subsequently failed to flee the shocks even when offered a means of escape⁹⁴. In rodent studies, it has been reported several times that escape deficits are reversible by antidepressants⁹⁴. On the down side, only a certain percentage of animals develop helplessness behaviour⁹⁵ and behavioural deficits persist only for 2-3 days⁹⁴.

2.1.6.2.4. The Unpredictable Chronic Mild Stress

Chronic Mild Stress (CMS) was first developed by Katz and Schmaltz. 1980⁹⁶ and Katz et al. 1981⁹⁷ and then modified by Willner¹¹. In the present study we used an unpredictable chronic mild stress (UCMS) model, which is a modified version of Willner's. UCMS procedure consists of sequentially applied series of relatively mild stressors, none of which is either necessary or sufficient to affect behaviour on its own: the essential feature of the model is variety and unpredictiveness⁹⁸. In this study we

applied an uncontrollable and unpredictable mild stressors which is detailed in the part of material and methods. This model has good face, predictive and constructs validity as we mentioned below.

Face validity

Face validity of an animal model point whether there are phenomenological similarities between the model and clinic⁸¹. As we mentioned before the main symptoms of depression are; the alterations in mood (difficult to test in animals), alteration in appetite and weight, changes in cognition, changes in the HPA axis, sleep disturbances, libido changes, poor hygiene, changes in motivation and drive, often coupled with decreased pleasure seeking or anhedonia. Almost all demonstrable symptoms of depression have been demonstrated by the UCMS model.

Initial studies with UCMS model were set out for modeling anhedonia which is a core symptom of the melancholic subtype of major depressive disorder (DSM IV). Anhedonia means the loss of interest in normally pleasurable and rewarding activities. It is a potentially useful endophenotype for modeling depression-related anhedonia in mice. Anhedonia is often measured as a decreased preference for sucrose, which is a solution that is usually preferred by rodents to water¹¹. UCMS can decrease the consumption of and preference for palatable solutions⁹⁹. It also reduces ventral tegmental self-stimulation¹⁰⁰, and place preference conditioned with food, sucrose solution, or amphetamine treatment¹⁰¹. These behavioural changes are prevented by concomitant treatment with antidepressants¹⁰².

In addition to decreasing responsiveness to rewards, UCMS also causes the appearance of many symptoms of major depressive disorder. Behavioural changes in animals exposed to UCMS include decreases in sexual^{103, 104}, aggressive¹⁰³ and investigate behaviors¹⁰⁵, and decreased locomotor activity. These alterations were seen during the dark phase of the light-dark cycle, which is the rodent's active period¹⁰⁶. In contrast, UCMS did not cause the appearance of an "anxious" profile in two animal models of anxiety; the elevated plus-maze and the social interaction test¹⁰⁷, suggesting that the behavioural changes are specific for depression.

Animals exposed to UCMS show a variety of sleep disorders characteristic of depression, including decreased rapid eye movement (REM) sleep latency, and increased number of REM episodes^{108, 109}.

There are several studies that show a variation of body weight¹¹⁰. It has been reported that the stressed mice gain weight slowly, leading to relative loss of body weight¹¹¹.

The mouse version of the model incorporates assessment of the animal's coat condition, which deteriorates in line with stress-induced deficits in grooming in an antidepressant-reversible manner^{33, 41}. This phenomenon has been considered analogous to the observation that depressed patients execute even the smallest tasks with great effort, often leading to poor personal hygiene.

Moreover, Song et al.¹¹² demonstrated that UCMS significantly decreased the cognitive performance of stressed mice in the water maze.

All these symptom profile of UCMS model are summarized in the Table 1. The only symptoms of depression that have not been demonstrated in animals exposed to UCMS are those uniquely human symptoms that are accessible to verbal enquiry¹¹³.

On the other hand, the UCMS procedure induces various neurochemical, neuroimmune and neuroendocrine alterations that resemble those observed in depressed patient¹¹⁴. This stress procedure induces endocrine alterations such as increased activity in the HPA axis, including adrenal hypertrophy⁹⁸ and corticosterone hypersecretion^{107, 115}. It reduces the weight of the thymus¹¹⁶ while it augments the heart rate¹¹⁷.

Table 1. Symptom profile of depression and UCMS. The left side of the table shows the symptoms required for major depressive episode; the right side of the table shows corresponding behavioural changes in rodents exposed to UCMS. N/A: non appropriated^{11,94}.

<i>DEPRESSION</i>	<i>UCMS</i>
Duration	
At least 2 weeks	Effects of UCMS persist for up to 3 months
Core symptoms	
Depressed mood	N/A
Loss of pleasure or lack of reactivity to pleasurable stimuli	Generalized decrease in responses to rewards
Markedly diminished interest/pleasure	Decreases in sexual and investigative behaviours
Other symptoms	
Significant weight loss	Weight loss typically around 5%
Insomnia or hypersomnia	Disrupted sleep patterns
Psychomotor agitation or retardation	Alterations in locomotor activity and motor function
Fatigue or loss energy	Reduced activity in home cage, decreased 'active waking' in EEG
Indecisiveness or diminished ability to think or concentration	Deficits in working and spatial memory
Difficulty performing even minor tasks, leading to poor personal hygiene	Poor coat condition, reduced grooming behaviour
Feelings of worthlessness or excessive or inappropriate guilt	N/A
Recurrent thoughts of death or suicide	N/A

Predictive validity

Predictive validity requires a behavioural response to antidepressants that involves a time delay in onset of action and specificity for only clinically active

antidepressants. The reversal of the UCMS-induced anhedonia typically requires 3-4 weeks of treatment, which closely resembles the clinical course of antidepressant action; a second parallelism with the clinic is that antidepressants act specifically in animals exposed to UCMS, but do not alter rewarded behaviour in non stressed animals¹¹. The antidepressants improve the behavioural changes induced by UCMS as well as neurochemical and neuroendocrine. The effects of a wide range of antidepressants, non-antidepressants and novel antidepressants were searched in the UCMS model (Table 2).

To summarize, a wide variety of antidepressant drugs, as well as electroconvulsive shock is active in counteracting the effects of UCMS in stressed but not in non-stressed animals. In addition, as predicted the time to show their effects closely mirrors the clinical action of these drugs in the UCMS model. These results suggest that the UCMS model is a convent model for the drug development.

Table 2. The effective and ineffective agents in the UCMS model are detailed.

<i>EFFECTIVE AGENTS</i>	<i>INEFFECTIVE AGENTS</i>
<i>Tricyclics</i>	<i>Anxiolytic</i>
Imipramine ¹⁰⁵	Chlordiazepoxide ¹¹¹
Desipramine ¹⁰³	
Amitriptyline ¹¹⁸	<i>Neuroleptics</i>
	Haloperidol ¹¹⁹
<i>SSRIs</i>	Chlorprothixene ¹¹⁹
Fluoxetine ^{33, 33}	Risperidone ¹¹
Sertraline ¹²⁰	
Citalopram ¹²¹	<i>Psychostimulant</i>
	Ampethamine ¹¹⁹
<i>NA reuptake inhibitor</i>	
Maprotiline ¹¹¹	<i>Opioid</i>
	Morphine ¹¹⁹
<i>MAO inhibitors</i>	
Moclobemide ¹²²	
Brofaromine ¹¹⁹	
<i>CRF antagonists</i>	
Antalarmin ⁴¹	
SSR 125543A ¹²³	
<i>Vasopressine rec. antagonist</i>	
SSR149415 ¹²⁴	
<i>Anti-manic agents</i>	
Lithium ¹²⁵	
Carbamazepine ¹²⁶	
<i>Electroconvulsive shock</i>	

Construct validity

The construct validity of the model derives from the evidence that UCMS causes alterations in the neurobiological system that participate the etiology of depression. UCMS modifies the regulation of monoaminergic receptors including 5-HT_{1A}¹²⁷ and β -adrenoreceptors¹⁰². It has been reported that UCMS elicits upregulation of β -adrenoreceptors and 5-HT₂ receptors in the brain¹⁰², which resembles the upregulations in human suicide victims¹²⁸. Thus, UCMS might be a suitable tool for testing the hypothesis implying the development of functional supersensitivity in monoaminergic neurotransmission.

Furthermore, the results obtain with UCMS is also convient with the hypothesis of elevated HPA axis in depression⁴¹.

Reliability

The procedure has two important drawbacks. One is the practical difficulty of carrying out UCMS experiments, which are labour intensive, demanding space, and of long duration. The other is that, while the procedure operates reliably in many laboratories especially for the mice studies, some of the commentators have still doubt. Although the UCMS model has been hampered by poor inter-laboratory reliability in rat studies⁸⁹, there are a lot of promising recent reports of its use in mice^{33, 41}.

Overall, the UCMS procedure appears to be at least as valid as any other animal model of depression. On balance it appears that the models such as learned helplessness, FST and TST which induce stress by a short term exposure of stressor is inconsistent with etiological factors which may induce a clinical depression. Indeed, one of the defining characteristics of depression is a persistence of depressed mood over a number of weeks. Furthermore, acute tests allow no opportunity for comparing the onset of action of compounds.

2.1.6.2.5. Early Social Isolation

Experience of stressful events during childhood has long been thought to contribute to the pathophysiology of emotional disorders¹²⁹. In this model animals are separated from their mothers at a young age (6-8 weeks)^{130, 131} that induce some persisting behavioural and HPA axis abnormalities in adulthood, which can be reversed by chronic antidepressants treatment.

2.1.6.3. Genetic Models

Genetic animal models of depression can be used to identify factors underlying predisposition to depression. In the first genetic model of depression, Flinders Sensitive line and Flinders Resistant line were selected for their differential hypothermic responses to an anticholinesterase agent¹³². Moreover, the Wistar-Kyoto rat exhibits several behavioural and hormonal abnormalities often associated with depression and it shows depressive-like behaviors in the relevant tests such as the learned helplessness and FST^{133, 134}. The development of models of depression based on the interaction between stress and genetic vulnerability appears plausible. This approach is a much more realistic mirror of the factors playing a role in human depression and may provide models which allow the definition of novel genes influencing behaviour⁹³.

On the other hand, knock out (KO) mice are frequently used to search the effect of a single gene. Especially despite the lack of highly selective ligands for a receptor subtype, such as the 5-HT_{1B} receptor subtype, made the analysis of pharmacological studies particularly difficult, and the use of KO mice can be helpful¹³⁵. Indeed, there are several studies realized by the 5-HT or NA receptor KO mice to investigate the participation of these receptors in the pathophysiology of depression. For example, Ramboz et al.¹³⁶ reported that 5-HT_{1A} KO mice exhibited a decreased immobility in the FST. Moreover, Cryan et al.⁸⁹ showed that NA-deficient mice failed to respond to the behavioural effects of antidepressants such as NA reuptake inhibitors desipramine and reboxetine and surprisingly the effects of SSRIs were also absent or attenuated in these mice.

2.1.6.4. Neuronal Deficits

Bilateral olfactory bulbectomy results in changes in behaviour, and in the endocrine, immune and neurotransmitter systems that simulates many of those seen in patients with major depression. The olfactory system in the rat forms a part of the limbic region in which the amygdala and hippocampus contribute to the emotional and memory components of behavior. However, the loss of olfaction alone, which results from bulbectomy, is not the major factor that contributes to the behavioural abnormalities, as peripherally induced anosmia does not cause the same behavioural changes. Thus, it would appear that bulbectomy causes a major dysfunction of the cortical-hippocampal-amygdala circuit that underlies the behavioural and other changes. These neuroanatomical areas also seem to be dysfunctional in patients with major depression. Chronic, but not acute, administration of antidepressants largely corrects most the behavioural, endocrine, immune and neurotransmitter changes that occur following bulbectomy¹³⁷.

2.1.7. The Pharmacology of Depression

Antidepressants enable to treat the symptoms of depression. Furthermore, the antidepressants also show efficacy in the treatments of other pathologies such as obsessive-compulsive troubles, pain and anxiety disorders.

2.1.7.1. History and Current Status of Antidepressant Treatments

Novel antidepressant therapies have emerged every decade since the late 1950s and early 1960s. The discovery of the antidepressant properties of MAOI compounds such as iproniazid, originally developed as anti-tuberculosis drug¹³⁸ was serendipitous and elucidation of possible mechanisms of action followed much later. The second generation of compounds such as the TCA, imipramine, were originally synthesized as analogues of the antipsychotic compound chlorpromazine and their antidepressant activity was also discovered in the clinic¹³⁹. Initially efforts were concentrated on compounds that enhanced NA concentrations. When it was shown that some of the

more successful compounds also had activity as reuptake blockers at the serotonin transporter, SSRIs took an attention¹⁴⁰.

Furthermore, there are also several antidepressants that selectively modify central noradrenergic function, such as mianserin (α_2 -presynaptic blockade) reboxetine and desipramine (selective NA reuptake inhibitors (NARIs)) or ‘dual action antidepressants’ venlafaxine and milnacipran that modify both central noradrenergic and serotonergic functions. Some authors find this latter group of ‘dual action antidepressants’ more efficient for the treatment of depression¹⁴¹.

While the available antidepressants (Table 3) have efficacy in the control of depressive symptoms, it is necessary to continue to search new compounds to improve in terms of latency of onset of therapeutic action, side effects profile and treatment of non-responders. Indeed, currently available antidepressants can take up to several weeks for their full therapeutic effect to be experienced by the patient¹⁴².

There has been an important improvement of knowledge about non-monoamine systems which can contribute to the pathophysiology of depression in animal models and some human evidence is also available¹⁴³⁻¹⁴⁵. However none of these discoveries have a utility in the treatment of depressed patients.

2.1.7.2. Monoamine Oxidase Inhibitors (MAOIs)

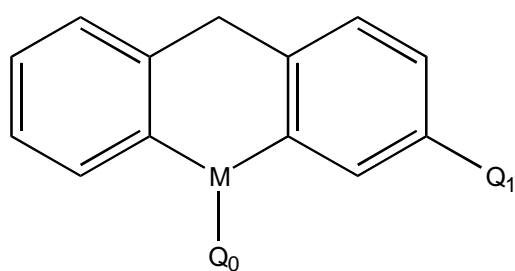
Drugs of the MAOI type were among the first to be introduced clinically as antidepressants but were largely superseded by TCA and other type of antidepressant with clinical efficacies that were considered better and with generally less side-effects. These drugs inhibit the MAO enzyme, which catabolizes the monoamines and they cause a rapid and sustained increase in the 5-HT, NA and DA content in the brain. There are two type of this enzyme; MAO-A and MAO-B. MAO-A is found primarily in the liver and gastrointestinal tract with some found in the monoaminergic neurons. MAO-A present in the liver is involved in the elimination of ingested monoamines such as dietary tyramine. Circulating monoamines such as adrenaline, NA and DA are inactivated when they pass through the liver rich in MAO-A. MAO-B is found in the brain and platelets. And the drugs classify belong to their effects on these different type of enzyme. The older MAOIs, such as phenelzine, isocarboxazid are considered non-

selective inhibitors while the newer MAOIs tend to be more specific inhibitors of either MAOI-A (Moclobemide) or MAOI-B. Additionally, the older MAOIs bind irreversibly to the enzyme, while the newer products are reversibly in a competitive equilibrium. They have a lot of unwanted effect such as hypotension, tremors, excitement, insomnia, weight gain and atropine-like side effects.

2.1.7.3. Tricyclic Antidepressants (TCAs)

TCA are named like this because of their molecular structure, which contains three rings of atoms (Fig.1). The main mechanism of action of TCA is to block the uptake of amines such NA, 5-HT and DA by nerve terminalis by competition for the binding site of the transport protein.

In non-depressed human subjects, TCA cause sedation, confusion and motor incoordination. These effects occur also in depressed patients in the first few days of treatment but tend to wear off in 1-2 weeks as the antidepressant effect develops. TCA produce a number of troublesome side effects, mainly because of interference with autonomic control. For example atropine-like effects include dry mouth, blurred vision, constipation and urinary retention, postural hypotension. The other common side effects are sedation, drowsiness and difficulty in concentrating.



Drug	R ₁	R ₂
Imipramine	-CH ₂ CH ₂ CH ₂ N(CH ₃) ₂	H
Desipramine	-CH ₂ CH ₂ CH ₂ NHCH ₃	H
Clomipramine	-CH ₂ CH ₂ CH ₂ N(CH ₃) ₂	Cl

Fig.1. The main structure of tricyclic antidepressants and some examples.

2.1.7.4. Selective Serotonin Reuptake Inhibitors (SSRIs)

SSRIs have replaced TCAs as the drugs of choice in the treatment of depressive disorders, mainly because of their improved tolerability and safety if taken in overdose¹⁴⁶. SSRIs block selectively the reuptake of 5-HT into the presynaptic nerve terminal, thereby enhancing 5-HT neurotransmission, which presumably results in their antidepressant effects.

SSRIs are the most widely used treatments for depression (e.g. citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline). In the US, these medications account for more than 80% of all the prescriptions written for the treatment of depression¹⁴⁷.

However even if they have least unwanted effects than the MAOIs and TCAs, SSRIs have been associated with a range of side effects including nausea and other gastrointestinal effects to impaired libido and sexual function¹⁴⁰. Finally a significant number of patients fail to respond to SSRIs treatment¹⁴⁸.

2.1.7.5. Dual Acting Antidepressants

Among the antidepressants, which are in the market, dual-acting antidepressants are the more recently discovered ones. The SNRIs, such as venlafaxine and duloxetine, act initially as 5-HT reuptake inhibitors, with NA reuptake blocking effects becoming more prominent with increasing dose. Another dual acting antidepressant is the noradrenergic and specific serotonergic antidepressant (NaSSA), such as mirtazapine.

Although the single-acting SSRIs are still the most commonly prescribed antidepressants, dual acting antidepressants are experiencing a surge in scientific and clinical interest, particularly because the newer dual acting antidepressants venlafaxine, duloxetine and mirtazapine have more slight side effects than the earlier dual-acting antidepressants, the TCAs and MAOIs. Venlafaxine and duloxetine may have an earlier onset of action, superior remission abilities, and better efficacy in treating the physical symptoms of depression than the SSRIs. This makes further research and clinical exploration of this class of antidepressants very exciting.

2.1.7.6. Atypical Antidepressants

Mianserin was the first atypical antidepressant discovered that lacks an inhibitory effect on the reuptake of monoamines and does not inhibit MAO¹⁴⁹. It blocks the presynaptic α_2 receptors.

The other atypical antidepressant is trazodone. It is a 5-HT₂ receptor antagonist which is effective like SSRIs and has relatively little risk in terms of cardiovascular safety¹⁵⁰.

In summary, although these drugs belong to different chemical groups and act at different sites of the monoaminergic neuron, they all share the property of modifying acutely the monoamine levels at the synapse.

2.1.7.7. Non-medication

25%-50% of depressed patients do not remit during short-term antidepressant trials. 10%-20% of patients do not achieve long-term benefit from existing treatments. Electroconvulsive therapy (ECT) is generally used in severely depressed patients for whom psychotherapy and medication are proving ineffective. It may also be considered when there is an imminent risk of suicide because ECT often has much quicker results than antidepressant remedies. But the loss of memory limits the use of this method. Transcranial magnetic stimulation (TMS) is the use of powerful rapidly changing magnetic fields to induce electric fields in the brain by electromagnetic induction without the need for surgery or external electrodes. TMS is a powerful tool in research for mapping out how the brain functions, and has shown promise for noninvasive treatment of most of disorders, including depression and auditory hallucinations.

On the other hand, vagus nerve stimulation (VNS) therapy is approved in United States for pharmacoresistant seizure disorders and the treatment-resistant depression. Vagus nerve projects to brain regions believed to be involved in the pathophysiology of mood disorders. It alters the neurotransmitter systems implicated in depression, for example it increases Homovallinic acid (HVA) and NA level in treatment resistant depression in patients. It alters the function of the brain regions that regulate mood such as amygdala, hippocampus¹⁵¹ (Table 3).

Table 3. Currently available antidepressants and non-medical treatments for the treatment of depression⁶³.

AD: Antidepressant, ECT: Electroconvulsive therapy, MAOIs: Monoamine oxidase inhibitors, MS: Magnetic Stimulation, NRIs: NA reuptake inhibitors, SSRIs: Selective serotonin reuptake inhibitors, SNRIs: Serotonin and NA reuptake inhibitors, VNS: Vagal nerve stimulation.

Type of Treatment	Mode Of Action	Examples
Medication		
Tricyclics	Inhibition of mixed NA and 5-HT reuptake	Imipramine, Amitryptiline
SSRIs	Inhibition of selective 5-HT reuptake	Fluoxetine, Citalopram
NRIs	Inhibition of selective NA reuptake	Reboxetine, Maprotiline
SNRIs	Inhibition of mixed NA and 5-HT reuptake	Venlafaxine, duloxetine
MAOIs	Inhibition of monoamine oxidase A (MAO _A) (Inhibition of MAO _B does not have antidepressant effects)	Moclobemide
Atypical AD	Unknown. Although these drugs have purported monoamine-based mechanisms, which are not necessarily involved in the drugs action	Mianserin, Trazodone
Non-medication		
ECT	General brain stimulation	
MS	General brain stimulation. A magnetic field is thought to affect the brain by inducing electrical currents and neuronal depolarization	
VNS	Alteration in brain regions and neurotransmitter systems	
Psychotherapies	Exact mechanism is uncertain, but is thought to involve learning new ways of coping with problems	Cognitive-behavioural and interpersonal therapy
Deep brain stimulation	Stimulation of brain regions (such as cingulate cortex)	

2.2. Serotonergic System

2.2.1. The Discovery of Serotonin

The discovery of 5-HT was rather serendipitous¹⁵². It was known to physiologists since the middle of the nineteenth century that after blood clots, the serum processes a substance, which constricts vascular smooth muscle so as to increase vascular tone. This vasoconstrictor substance was often referred to as “vasotonin”. Around the turn of the twentieth century, platelets were identified as the source of this substance. Later on in the 1940’s, Page and coworkers isolated and characterized this ‘tonic’ substance in ‘serum’ and named it ‘serotonin’¹⁵³. The chemical structure of serotonin was found to be 5-Hydroxytryptamine. The identification of 5-HT in the CNS was made by Twarog and Page¹⁵⁴. 5-HT has been implicated in almost every conceivable physiologic or behavioural function such as aggression, appetite, cognition, emesis, endocrine function, gastrointestinal function, motor function, neurotrophism, perception, sensory function, sex, sleep, and vascular function^{155, 156}.

2.2.2. The Synthesis, Storage, Release and Metabolism of Serotonin

5-HT is a biogenic amine present in a variety of organisms ranging from worms to humans¹⁵⁷. It acts as a neurotransmitter and is found in a wide variety of sites in the central and peripheral nervous system¹⁵⁸. 5-HT is a derivative of the naturally occurring amino acid L-tryptophan (LT) which is intrinsically fluorescent¹⁵⁹ and found in foods such as bananas, pineapples, plums, turkey and milk. The enzyme tryptophan hydroxylase adds a hydroxyl group to tryptophan's benzene ring at position 5, creating 5-hydroxytryptophan (5-HTP). This reaction is the rate limiting step of the 5-HT synthesis and it requires the existence of cofactors such as oxygen and tetrahydrobiopterine. Another enzyme, amino acid decarboxylase, then removes a carboxyl group from 5-hydroxytryptophan, forming 5-hydroxytryptamine, which is more commonly known as 5-HT. This last reaction uses pyridoxal-5-phosphate (the active form of B6 vitamin) as coenzyme (Fig.2). Tryptophan hydroxylase can be inhibited by numerous factors, including stress, insulin resistance, vitamin B6 deficiency, and insufficient magnesium. In addition, these same factors can increase the

conversion of LT to kynurenine via tryptophan 2, 3-dioxygenase, making LT unavailable for 5-HT production.

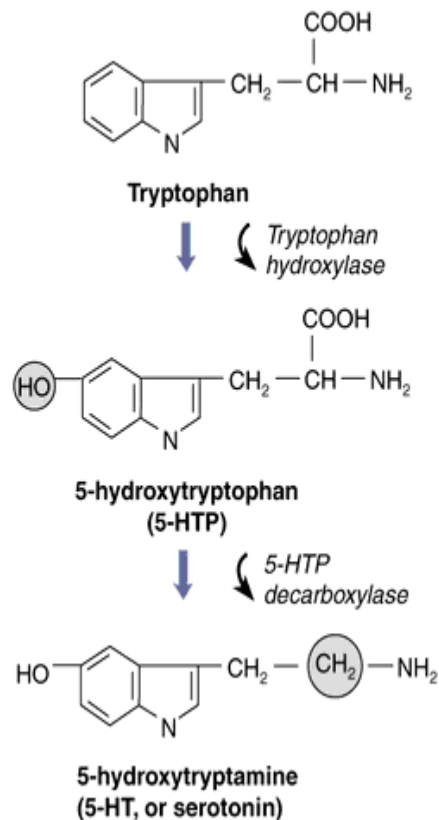


Fig.2. The synthesis of serotonin

5-HT stores in reserpine-sensitive vesicles. The release mechanism of 5-HT is basically the same as those at other transmitters (Fig.3). In the synaptic cleft, 5-HT can act on both postsynaptic receptors that convey information to other neurons and presynaptic receptors that regulate 5-HT synthesis and release.

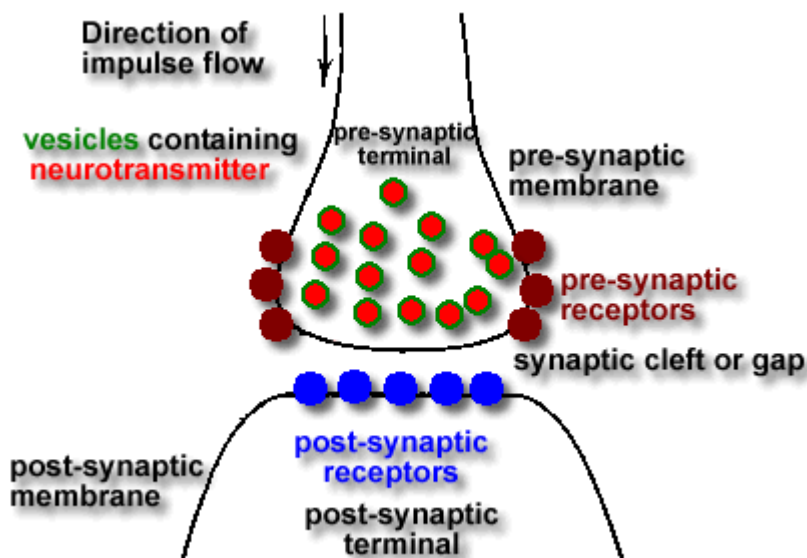


Fig.3. The process of 5-HT release.

Inactivation mainly is due to reuptake through a membrane carrier that transports 5-HT from the synaptic cleft back into the neuron. On the second hand, intraneuronal breakdown is accomplished by the enzyme MAO, resulting in the metabolite 5-hydroxyindolacetic acid (5-HIAA). At least 2 forms of MAO, differing in substrate affinities and inhibitors, are known. MAO-A has highest affinity for 5-HT and norepinephrine, whereas MAO-B is more active in deaminating benzylamine and phenylethylamine¹⁶⁰.

These multiple steps in synthesis, storage, release and metabolism of the 5-HT allow for multiple pharmacological interventions¹⁶¹.

All 5-HT used by brain cells must be made within the neurons, since 5-HT cannot cross the blood-brain barrier. However the amino acid tryptophan and its metabolite 5-hydroxytryptophan cross the blood-brain barrier.

2.2.3. Central Serotonergic Pathways

Central 5-HT system is organized into two subsystems, i.e., a rostral (superior) division with cell bodies localized in the midbrain and rostral pons, providing

projections to the forebrain, and a caudal division (inferior) located primarily in the medulla oblongata with descending projections to the spinal cord and brainstem nuclei. The rostral system consists of four main nuclei: caudal linear nucleus, median raphe nucleus (MRN), dorsal raphe nucleus (DRN) and suprallemniscal region. The inferior group consists of five main nuclei: nucleus raphe obscurus, nucleus raphe magnus, ventrolateral medulla, intermediate reticular nuclei and area postrema¹⁵⁸. It is important to note that although the serotonergic cell groups closely match the raphe nuclei, some serotonergic neuronal cell bodies are found outside the raphe nuclei, and not all the cell bodies within the raphe nuclei are serotonergic¹⁶². The DRN is the largest of the brainstem serotonergic nuclei containing about 50% of 5-HT neurons in the rat CNS and 50-60% in human CNS¹³(Fig.4). In this study, we were interested in serotonergic system and particularly DRN, for this purpose the afferents and efferent's pathways of raphe nucleus were detailed above.

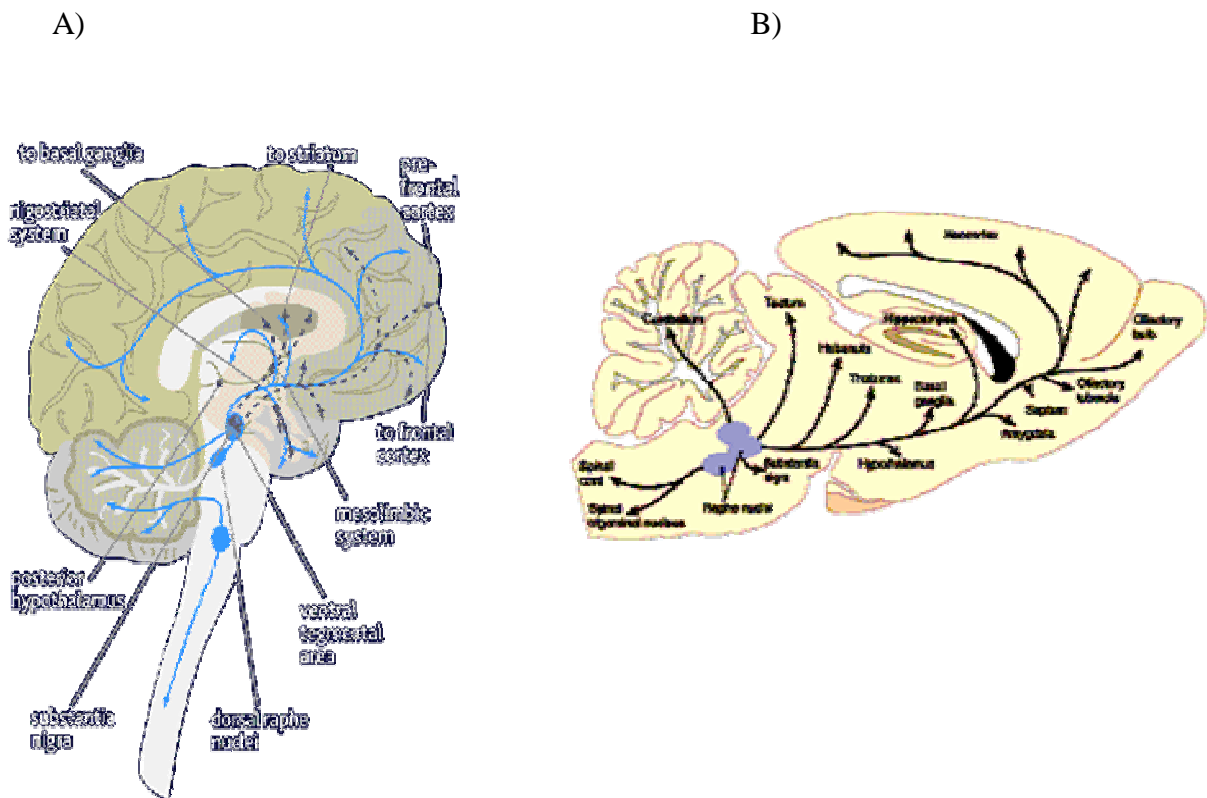


Fig.4. The serotonergic pathways in the human (A) and rat (B) brain.

2.2.3.1. Afferents to Midbrain 5-HT Nuclei

The raphe nucleus receives the afferents from multiple origins that have an important role in the mood disorders. The main sources of the 5-HT fibers reaching the DRN arise either from the DRN itself or the MRN¹⁶³. The second important afferent area to the raphe is the lateral habenula. The serotonergic neurons also receive inputs from locus coeruleus, hypothalamus, substantia nigra and ventral tegmental area, periaqueductal gray, nucleus of solitary tract, amygdala, stria terminalis, nucleus accumbens¹⁶⁴ and by these afferent, there are several neurotransmitters implied such as NA, DA, acetylcholine, GABA etc. Multiple afferent fibers are also immunoreactive for different neuropeptides, such as β -endorphin, substance P, neurotensin fibers, cholecystokinin (CCK) fibers, ACTH and vasoactive intestinal peptide (VIP) fibers¹³.

2.2.3.2. Efferent Pathways and Terminal Projections Areas

Jacobs and Azmitia.¹⁵⁸, mapped five main ascending pathways (into the forebrain) and three descending pathways (into the spinal cord) that provide redundant entry routes into terminal areas.

The serotonergic fibers projecting to the forebrain, including limbic areas (prefrontal cortex, cingulate cortex, entorhinal cortex, hippocampus, nucleus accumbens, ventral pallidum, amygdala and anterior hypothalamus) originate mainly in the superior group of raphe nuclei¹⁶⁴. In the rat, the largest ascending pathway is the medial forebrain bundle, which carries fibers from the MRN and the DRN to a wide range of target areas in the forebrain. In primates, the largest pathway appears to be the dorsal raphe cortical tract, which enters cortex through the internal capsule network.

There are at least three main pathways into the spinal cord. The nucleus raphe obscurus innervates the ventral horn of the spinal cord via the descending pathways like the posterior fasciculus of the spinal cord. The nucleus raphe magnus innervates the dorsal horn. The last major input into the spinal cord comes from the ventral lateral medullary 5-HT neuron. This fibers use the lateral fasciculus of the spinal cord to innervate the lateral horn.

2.2.4. Central Serotonin Receptors

5-HT exerts its diverse actions by binding to distinct cell surface receptors which have been classified into many groups on the basis of their pharmacological responses to specific ligands, sequence similarities at the gene and amino acid levels, gene organization and second messenger coupling pathways¹⁶⁵.

The 5-HT systems are subdivided in seven subtypes, named 5-HT₁, 5-HT₂, 5-HT₃, 5-HT₄, 5-HT₅, 5-HT₆ and 5-HT₇ receptors.

Most of the 5-HT receptors, except the 5-HT₃ receptor which is a ligand-gated ion channel, belong to the large family of seven transmembrane domain G-protein coupled receptors¹⁶⁶ that couple to and transduce signals via guanine nucleotide binding regulatory proteins (G-proteins)¹⁶⁷.

2.2.4.1. The 5-HT₁ Receptor Family

The 5-HT₁ receptor subset is composed of at least five receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}) that are linked to inhibition of adenylyl cyclase activity or to regulation of potassium (K⁺) and calcium (Ca²⁺) channels.

The 5-HT_{1A} receptor is the most extensively studied of the 5-HT receptors¹⁶⁸. These receptors are located both pre- and postsynaptically. 5-HT_{1A} presynaptic receptors are located on both somas and serotonergic neuron dendrites in raphe nuclei (especially DRN which is known as somatodendritic receptors)^{169, 170} and in postsynaptic regions such as the hippocampus, thalamus, hypothalamus and amygdala¹⁷¹. It is believed that somatodendritic 5-HT_{1A} autoreceptors in the DRN causes inhibition of the firing rate of 5-HT neurons and therefore inhibit 5-HT release at the nerve terminals.

In both locations, 5-HT_{1A} receptor activation leads to neuronal hyperpolarization via the opening of an inwardly rectifying K⁺ conductance and a reduction of firing rate. They are thought to be involved in temperature regulation. They are also found in regions of the CNS associated with mood and anxiety such as hippocampus and amygdala.

5-HT_{1B/1D} receptors are expressed throughout the mammalian central nervous system. These receptors are located in the axon terminals of both serotonergic and non-

serotonergic neurons, where they act as inhibitory autoreceptors or heteroreceptors, respectively. 5-HT_{1B/1D} receptors inhibit the release of a range of neurotransmitters, including 5-HT, GABA, acetylcholine, and glutamate¹⁷². These receptors unlike the somatodendritic receptors can modify 5-HT release without altering 5-HT neuron firing activity¹⁷³.

Both 5-HT_{1A} and 5-HT_{1B} receptors have an important role in the mood disorders, which were detailed below.

2.2.4.2. The 5-HT₂ Receptor Family

Three receptor subtypes constitute the 5-HT₂ classes: 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}. These receptors are linked to activation of phospholipase C.

5-HT_{2A} and 5-HT_{2C} receptors have widespread distribution and function in CNS. 5-HT_{2A} receptors are enriched in forebrain regions such as neocortex, olfactory tubercle, as well as in several nuclei arising from the midbrainstem¹⁷⁴. The 5-HT_{2C} receptor, which is very similar in sequence and pharmacology to the 5-HT_{2A} receptor, is expressed abundantly in the choroid plexus where it regulates transferring¹⁷⁵ and cerebrospinal fluid productions¹⁷⁶.

5-HT_{2A} and 5-HT_{2C} receptor antagonism is a property of certain antipsychotic and antidepressants drugs¹⁷⁷.

5-HT_{2B} receptors have a restricted expression in the CNS. They have an important role in the embryogenesis and in the peripheral organs.

2.2.4.3. The 5-HT₃ Receptor Family

The 5-HT₃ receptor is a member of the superfamily of ligand-gated ion channels, a family that also includes the neuronal nicotinic acetylcholine receptors, and the inhibitory neurotransmitter receptors for GABA (both GABA_A and GABA_C receptors) and glycine¹⁷⁸. Two subtypes of receptors; 5-HT_{3A} and 5-HT_{3B} compose this receptor family. Receptors of the 5-HT₃ classes first were recognized in the peripheral autonomic system. They are also expressed in brain within the area postrema and nucleus tractus solitarius, where they couple to potent depolarizing responses that show rapid

desensitization to continued 5-HT exposure. 5-HT₃ receptor leads to enhanced Na⁺ and K⁺ currents.

These receptors play a prominent role in chemotherapy- and radiotherapy-induced emesis.

2.2.4.4. 5-HT₄ Receptors

The serotonin 5-HT₄ receptor mediates many physiological effects in the central nervous system. The recent molecular identification of 5-HT₄ receptors and the development of selective 5-HT₄ receptor ligands have led to many important new insights into the signaling pathways and the physiological roles of these G protein-coupled-receptors in neurons. With respect to neurodegenerative disorders, it is suggested that 5-HT₄ agonists may represent a new avenue for the treatment of Alzheimer's disease¹⁷⁹.

2.2.4.5. The 5-HT₅ Receptors Family

The 5-HT₅ receptor family consists of two members designated as 5-HT_{5A} and 5-HT_{5B}. To date the 5-HT_{5A} receptor has been identified in the mouse, rat, and human. The 5-HT_{5B} receptor also is expressed in the mouse and rat, but not in the human. Both receptors are essentially limited in distribution to the CNS, although the 5-HT_{5A} receptor has also been found on neurons and neuronal-like cells of the carotid body. Within the CNS, the 5-HT_{5A} receptor shows a relatively broad distribution, while the 5-HT_{5B} receptor has a very restricted distribution. The 5-HT_{5A} receptor has been demonstrated to couple to G proteins, and the primary coupling appears to be through Gi/o to inhibit adenylyl cyclase activity. The 5-HT₅ receptors have not been extensively characterized pharmacologically. Both receptors show their highest affinity for Lysergic acid diethylamide (LSD), which appears to act as a partial agonist at the 5-HT_{5A} receptor. Nothing is known about the role of the 5-HT_{5B} receptor in vivo. A mouse line has been developed where the 5-HT_{5A} receptor has been knocked out (KO) and these animals have been shown to have a diminished response to LSD-induced increase in

locomotion. The 5-HT₅ receptors remain the least studied and understood of the 5-HT receptor subtypes¹⁸⁰.

2.2.4.6. 5-HT₆ Receptors

In the rat brain, 5-HT₆ receptor mRNA, detected by Northern blot analysis, in situ hybridization, or RT-PCR, has been localized to the olfactory tubercle, nucleus accumbens, striatum, hippocampus, and cerebral cortex.

The physiological role of 5-HT₆ receptors in the human brain is not yet clearly understood. But, the localization of 5-HT₆ receptors to both basal ganglia and limbic structures suggests that this receptor may participate in the serotonergic control of motor function, mood-dependent behavior, depression, and cognition¹⁸¹.

2.2.4.7. 5-HT₇ Receptors

The 5-HT₇ receptor was among a group of 5-HT receptors that were discovered using targeted cloning strategies 12 years ago. This receptor is a seven-transmembrane-domain G-protein-coupled receptor that is positively linked to adenylyl cyclase.

The distribution of 5-HT₇ receptor shows that the highest receptor densities are present in the thalamus and the hypothalamus and significant densities are present in the hippocampus and the cortex¹⁸². It has been reported that 5-HT₇ receptor-selective ligands might prove therapeutically useful for the treatment of psychiatric disorders. Furthermore, there is evidence to suggest that this receptor may play a role in other CNS disorders including, anxiety, cognitive disturbances and also migraine probably via both peripheral and central mechanisms¹⁸³ (Table 4).

Table 4. The main properties of central 5-HT receptors

Receptors	Agonists	Antagonists	Localizations	Functions
5-HT_{1A}	8-OH-DPAT Gespiron, Buspirone Ipsapirone	WAY 100635 WAY 100135 NAD-299,p- MMPI, Spiperone Pindolol	Raphe nucleus, Hippocampus, Septum, Amygdala	Anxiety, Depression Sexual and eating behaviour, Cognition, Locomotion, Thermoregulation, Alzheimer
5-HT_{1B}	5-CT, Sumatriptan, L694247, RU 24969	GR 127935 SB-224289 SB-216641	Striatum, Cortex, Globus pallidus, Substantia nigra	Locomotion, Analgesia, Anxiety, Depression Aggressive and eating behaviour, Thermoregulation
5-HT_{1D}	Sumatriptan	GR 127935 BRL-15572	Hippocampus, Substantia nigra, Cortex	Migraine
5-HT_{1E}	-	-	Hippocampus, Cortex, Amygdala	-
5-HT_{1F}	LY 334370 LY 334864	-	Cortex, Striatum Hippocampus, Thalamus, Olfactory bulb	Migraine
5-HT_{2A}	DOI	Ketanserin, MDL 100907, Spiperone	Cortex, Striatum Hippocampus, Olfactory bulb	Thermoregulation, Anxiety, Schizophrenia
5-HT_{2B}	BW723C86	SB204741 SB200646	Hippocampus, Hypothalamus, Cerebellum, Septum Amygdala	Anxiety, Migraine, Eating behaviour
5-HT_{2C}	Ro600175	RS-102221 SB 242084 Mesulergine	Cortex, Substantia nigra, Nucleus accumbens, Amygdala, Hippocampus	Anxiety, Locomotion, Cognition, Thermoregulation, Eating behaviour

Table 4. (Continue) The main properties of central 5-HT receptors

Receptors	Agonists	Antagonists	Localizations	Functions
5-HT₃	m-CPBG SR 57227	Ondansetron Granisetron Tropisetron	Tractus solitari, Hippocampus, Amygdala	Anxiety, Locomotion, Cognition, Schizophrenia
5-HT₄	ML 10302, BIMU 8	SB204070, GR113808	Hippocampus, Substantia nigra, Olfactory bulb	Anxiety, Cognition
5-HT_{5A}	-	-	Olfactory bulb, Hippocampus, Cerebellum	Locomotion
5-HT_{5B}	-	-	Hippocampus, Habenula	-
5-HT₆	-	Ro 630563 SB 357134	Hippocampus, Hypothalamus, Thalamus, Amygdala, Cortex	Depression
5-HT₇	-	SB 258719	Hippocampus, Hypothalamus, Cortex, Amygdala	Circadian rhythm, Epilepsy

2.2.5. Serotonergic System and Mood Disorders

The involvement of serotonergic neurotransmission in a number of neurological and psychiatric conditions has been widely documented in recent years such as schizophrenia, migraine, depression, suicidal behaviour, infantile autism, eating disorders and obsessive compulsive disorder¹⁵⁹. In parallel, anatomical and developmental studies of the mammalian serotonergic system, including in humans, have significantly enlarged our understanding on its functional organization.

Among other neurotransmitters related with mood disorders, 5-HT is one of the most studied at both clinical and preclinical.

2.2.5.1. Biochemistry of 5-HT and Depression

Several observations point to a deficit in 5-HT metabolism in major depression. In the early 1960s, lowering of urinary and CSF 5-HT and/or its metabolite 5-HIAA was found in depressive patients. Both animal and human (postmortem) studies¹⁸⁴ have revealed a close correlation between brain and CSF 5-HIAA. Furthermore, the 5-HIAA concentrations in the brain is a large extent a function of 5-HT metabolism. Therefore, CSF 5-HIAA can be considered as an indicator of 5-HT metabolism. The 5-HT content in brains of suicide victims was found to be low as compared with controls. In addition, there was some evidence that there was a decrease in 5-HIAA in the suicide group¹⁸⁵.

A second group of data is derived from the so-called tryptophan-depletion strategy. As it was mentioned before, tryptophan is an essential amino acid and the precursor of 5-HT. A shortage of tryptophan by amino acids that can compete with tryptophan for the same transport mechanism in the blood-brain barrier leads to the occurrence of mood lowering¹⁸⁶. Furthermore, some depressed patients exhibit reduced tryptophan availability in plasma¹⁸⁷, reduced increase in plasma 5-HTP after treatment with L-tryptophan¹⁸⁸ and decreased uptake of 5-HTP across the blood-brain barrier¹⁸⁹. These data also suggest a defect in the synthesis of 5-HT in the depressed patients.

2.2.5.2. The 5-HT Receptors and Depression

Besides, the impairments in the biochemistry of 5-HT, receptor disturbances are also very important points explaining the participation of the serotonergic system in the mood disorders.

The 5-HT_{1A} receptor agonists¹⁹⁰ and antagonists¹⁹¹ represent a major class of molecules with potential therapeutic effects in anxiety-or stress related disorders. As a result, the 5-HT_{1A} receptors serve as an important target in the development of therapeutic agents enabling to treat neuropsychiatry disorders such as anxiety and depression. A substantial amount of evidence shows that stress can alter 5-HT_{1A} receptor. For instance, chronic social stress and forced swimming stress decrease 5-HT_{1A} density in the hippocampus¹⁹². On the clinical front, 5-HT_{1A} receptor levels have been shown to be altered in patients suffering from major depression¹⁹³. Positron emission tomography (PET) studies provided direct evidence for 5-HT_{1A} receptor

pathology in depression. A widespread reduction in 5-HT_{1A} receptor binding was reported in patients with major depression^{194, 195}.

As well known, most antidepressant drugs increase the concentration of 5-HT in central synapses by preventing its reuptake. However, this increase is offset by a negative feedback operating at the level of the 5-HT containing cell. Because SSRIs markedly increase the 5-HT concentration in the vicinity of 5-HT containing cells in the midbrain raphe nuclei, which leads to activation of 5-HT_{1A} autoreceptors, inhibition of cell firing and the reduction of 5-HT release in the forebrain¹⁹⁶. For that reason, although antidepressants induce reuptake blockade quickly, the onset of significant symptom relief typically occurs only after 2-3 weeks of treatment¹⁹⁷. With 2-3 weeks of continued administration of SSRIs, 5-HT_{1A} autoreceptors become desensitized which results in normalization of cell firing and enhancement of 5-HT transmission. This time correlates with the time of onset of the antidepressant effect^{198, 199} (Fig.5).

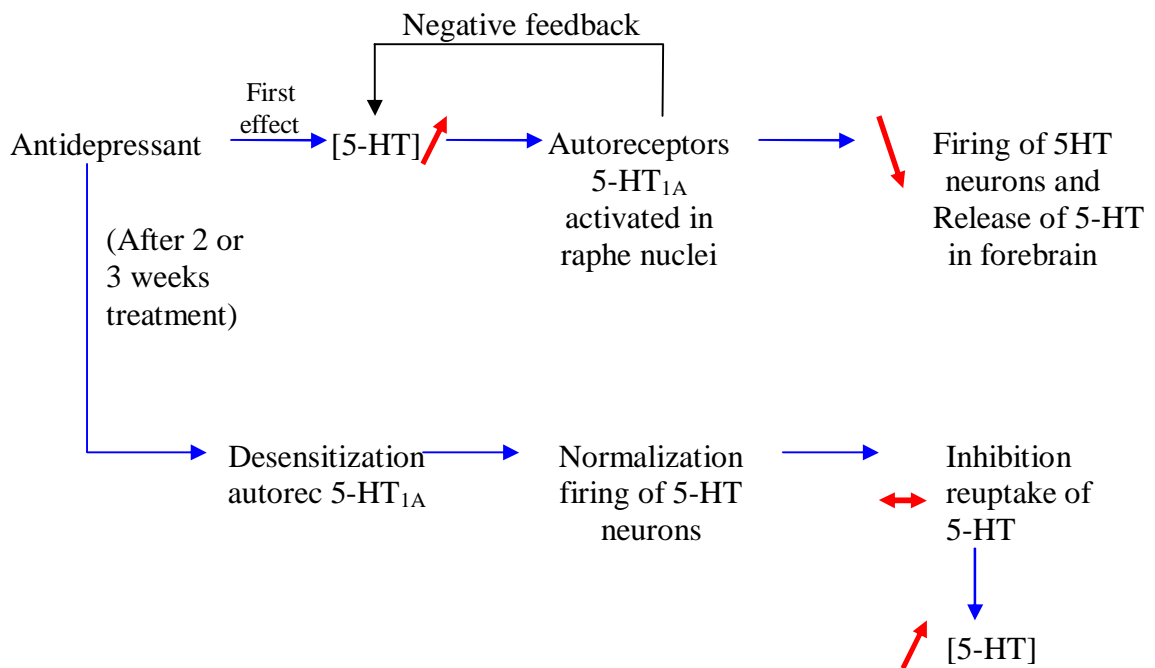


Fig.5. The effects of antidepressants on the 5-HT receptors.

Opposing effects of SSRIs on the concentration of 5-HT in forebrain synapses result from the inhibition of 5-HT reuptake at two distinct anatomical sites. Inhibition of reuptake in forebrain nerve terminals increases the extracellular 5-HT concentration. The concurrent inhibition in the midbrain raphe nucleus also increases 5-HT (more than in the forebrain), which then activates 5-HT_{1A} autoreceptors and reduces both 5-HT cell firing and 5-HT release by forebrain axes. The activation of terminal 5-HT_{1B} receptors also reduces 5-HT release. Subsequently, it was proposed that 5-HT_{1A} receptor antagonists such as pindolol could accelerate (and perhaps augment) the clinical effects of antidepressants by preventing this negative feedback²⁰⁰. This would enable a more rapid increase of synaptic 5-HT, preventing the inhibition of 5-HT release observed in microdialysis studies²⁰⁰. This antagonism mimics the 5-HT_{1A} receptor desensitization produced by the prolonged administration of antidepressants¹⁹⁹ (Fig.6).

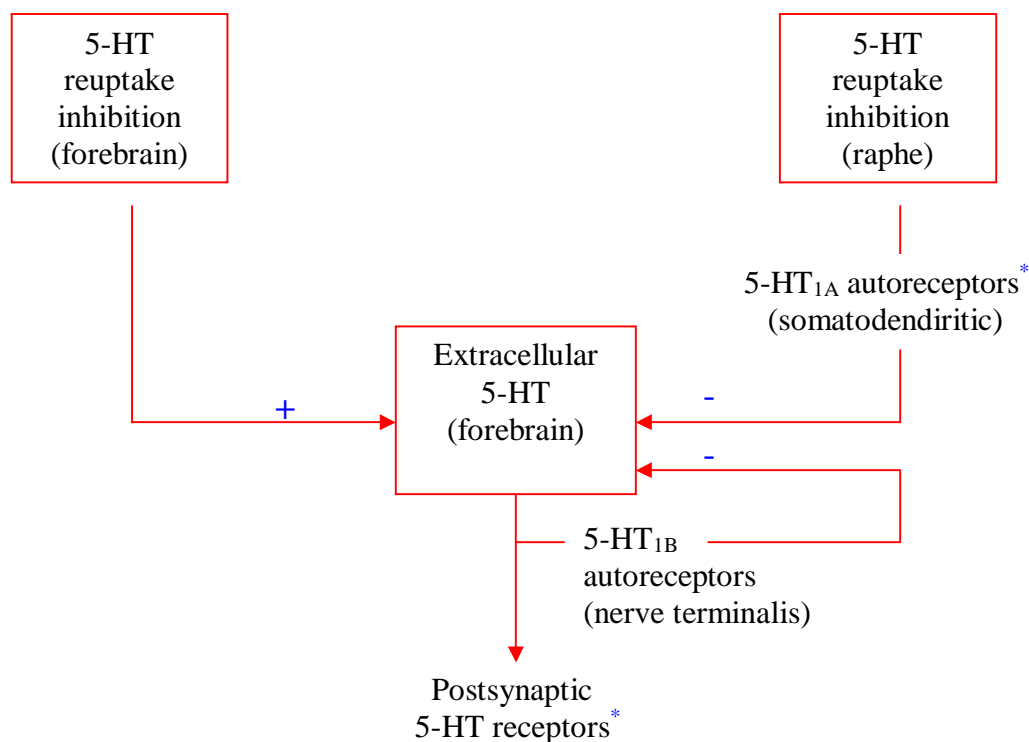


Fig.6. The effects of SSRIs and the 5-HT_{1A} receptor antagonist pindolol in the forebrain and raphe nucleus. Asterisks denote the possible sites of action of pindolol in human brain.

Selective 5-HT_{1A} receptor agonists produce responses in rodent behaviours, such as the FST and TST, that are similar to those produced by conventional antidepressants and the effects of antidepressants are blocked by 5-HT_{1A} receptor antagonists²⁰¹.

Studies have also implicated the 5-HT_{1B} receptor in antidepressant behavioural effects in rat and mice²⁰², although few compounds are available that substantially discriminate 5-HT_{1B} receptors from other 5-HT receptors.

Terminal autoreceptors (5-HT_{1B} receptors) further limit the increase in synaptic (extracellular) 5-HT produced by SSRIs (Fig.6). Gardier and Bourin.¹³⁵ suggested that 5-HT_{1B} autoreceptors limit the effect of a single administration of SSRIs particularly in the hippocampus, while the activation of postsynaptic 5-HT_{1B} receptors mediates the antidepressant activity of SSRIs (Fig.6).

In recent years, a number of open-label and placebo-controlled studies have suggested that atypical antipsychotic drugs and some antidepressants (e.g. mirtazapine and mianserin) also augment the clinical response to SSRIs in treatment-resistant patients^{203, 204}. One common feature of these agents is their ability to block the 5-HT₂-mediated responses. Likewise, many antidepressants downregulate 5-HT_{2A} receptors after repeated treatment. All together, these observations support a role for 5-HT_{2A} receptors in antidepressant drug action.

The recent development of 5-HT receptor KO mice has permitted the study of behavioural effects and drug responses in animals that have genetic deletion of targeted 5-HT receptors. For example, Santarelli et al.³³ showed that deletion of the 5-HT_{1A} receptor resulted in a blockade of both the behavioural and neurogenic effects of fluoxetine. On the other hand, in the recent years the polymorphism studies opened another perspective to improve our knowledge about depression and serotonergic system. Indeed, two polymorphic regions have been identified in the SERT promoter and it has also been demonstrated their implication in mood disorders²⁰⁵.

2.3. Noradrenergic System

2.3.1. The Synthesis, Storage, Release and Metabolism of Noradrenaline

NA was first identified in 1946 by the Swedish scientist Ulf Svante von Euler-Chelpin. He also found that NA is stored within nerve fibers themselves.

The biosynthetic pathway for NA synthesis is shown in Fig.7. The metabolic precursor for NA is L-tyrosine, an aromatic amino acid present in the body fluids, which are taken up by adrenergic neurons. Tyrosine hydroxylase, a cytosolic enzyme that catalyses the conversion of tyrosine to dihydroxyphenylalanine (dopa), is found only in catecholamine-containing cells. It is a rather selective enzyme; unlike other enzymes involved in catecholamine metabolism, it does not accept indole derivatives as substrates and so is not involved in 5-HT metabolism. This first hydroxylation step is the main control point for NA synthesis. The next step, conversion of dopa to dopamine, is catalyzed by dopa decarboxylase. Finally, the dopamine β -hydroxylase, which is located in synaptic vesicles, converts DA into NA.

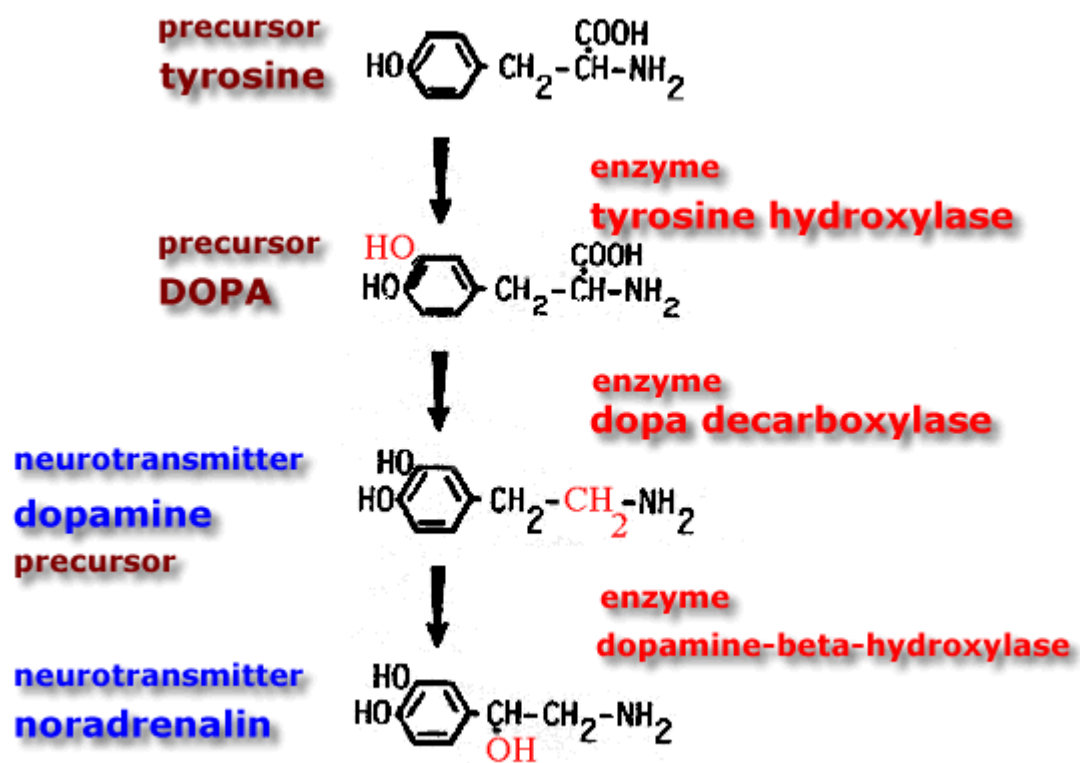


Fig.7. The synthesis of noradrenaline.

Most of the NA in nerve terminals or chromaffin cells is contained in vesicles. The process of NA release is basically the same as those at other chemically transmitting synapse. Depolarization of the nerve terminal membrane is induced by the opening of the calcium channels. The entry of Ca^{+2} promotes the fusion and discharge of synaptic vesicles. NA by acting on presynaptic receptors, can regulate its own release. This is believed to occur physiologically, so that released NA exerts a local inhibitory effect on the terminals from which it came (autoinhibitory feedback mechanism). This operates through α_2 and β_2 -adrenoceptors.

The action of released NA is terminated by reuptake of the transmitter into the noradrenergic nerve terminals by neuronal and extraneuronal uptake. In addition, it is metabolized by MAO and catechol-O-methyl transferase (COMT) enzyme (Fig.8). By the catabolism of MAO, 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylglycol, 3,4 dihydroxymandelic acid and 3-methoxy-4-hydroxymandelic acid are obtained whereas normetanephrine is derivated via COMT enzyme. Among these metabolites, MHPG is a major metabolite of NA, which is derived from the release of NA from peripheral and central noradrenergic neurons. Substantial evidence suggests that MHPG levels could be a possible index of central noradrenergic function.

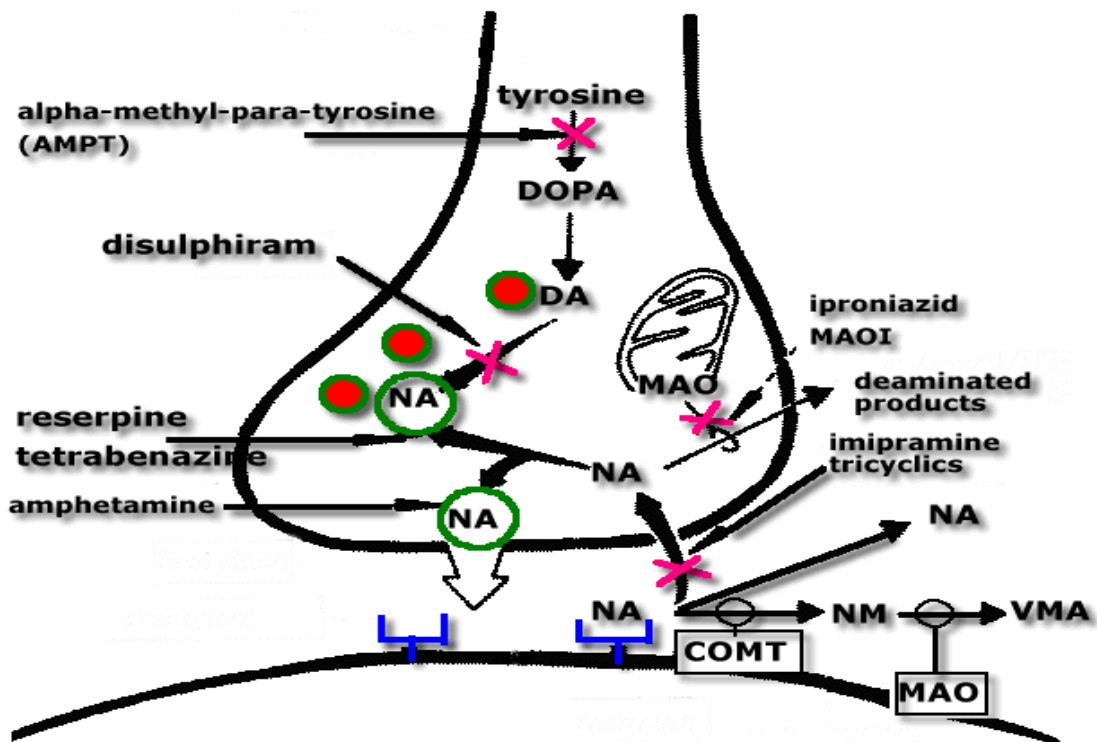


Fig.8. The catabolism of NA.

2.3.2. Central Noradrenergic Pathways

NA containing axons are distributed widely throughout the CNS, suggesting a prominent role of this neurotransmitter in CNS function and behaviour. A majority of brain noradrenergic neurons are brainstem nucleus located in the locus coeruleus (corresponding to A4+A6 cell groups as described by Dahlström and Fuxe²⁰⁶) (LC). Most of the other noradrenergic neurons are clustered in a region described as the lateral tegmental area (corresponding to A1, A2, A5 and A7 cell groups as described by Dahlström and Fuxe^{206, 207}). The LC resides on the dorsal wall of the upper pons, under the cerebellum in the caudal midbrain, surrounded by the fourth ventricle. The LC innervates virtually the entire CNS including thalamus, cortex, hippocampus, hypothalamus, cerebellum, amygdala, telencephalon and cortex, an exception to this

being the basal ganglia (striatum, globus pallidus), which are nearly devoid of noradrenergic input (Fig.9).

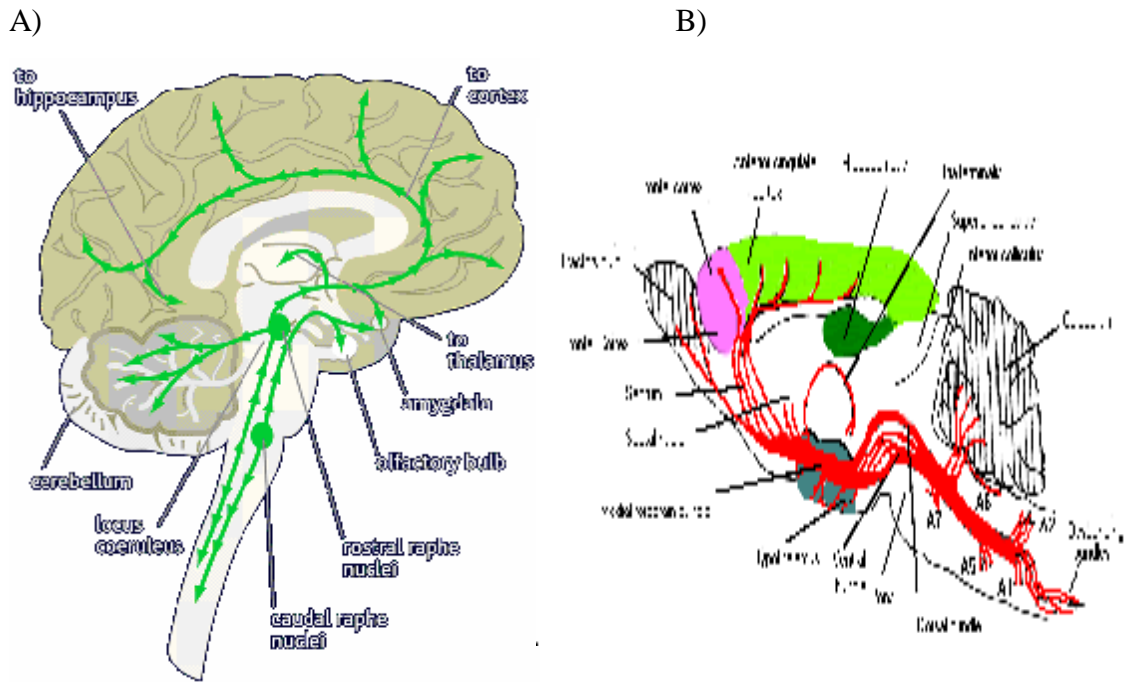


Fig.9. The noradrenergic pathways in human (A) and in rat (B) brain.

2.3.2.1. Afferent Pathways

The LC receives afferents from the hypothalamus. The cingulate gyrus and the amygdala also innervate the LC, allowing emotional pain and stressors to trigger noradrenergic responses. The cerebellum and afferents from the raphe nuclei also project to the LC, particularly the raphe pontis and raphe dorsalis. The LC receives inputs from a number of other brain regions such as medial prefrontal cortex, nucleus paragigantocellularis, nucleus prepositus hypoglossi, lateral hypothalamus.

Many neurotransmitters are involved in the innervation of the LC and are found in the neurons of the two major LC afferents (nucleus paragigantocellularis and nucleus prepositus hypoglossi), including adrenaline, glutamate, enkephalin, CRF, substance P, 5-HT and GABA²⁰⁸.

2.3.2.2. Efferent Pathways

The LC is the site of the majority of noradrenergic projections throughout the neuroaxis innervating areas such as the cortex, hippocampus and amygdala as well as the cerebellum and spinal cord; this ascending projection system is also referred to as the dorsal noradrenergic bundle. Efferent's from the lateral tegmentum form the ventral noradrenergic bundle and have less extensive projections. They provide predominant innervation of the hypothalamus and also innervate areas of the septum and the extended amygdala nuclei including the bed nucleus of the stria terminalis.

The connection between the dorsal raphe and the LC is particularly intriguing since the efferent projections of these two monoamine systems demonstrate considerable overlap in forebrain circuits. Not only do NA and 5-HT neurons interact at the level of their cell bodies in the raphe nuclei and LC, but also they both project to the same neurons in the forebrain²⁰⁹. Moreover, in rat cortex NA and 5-HT exert complementary modulatory actions on neuron responses²¹⁰.

2.3.3. Adrenergic Receptors

Three families of adrenergic receptors have been described in the CNS; α_1 , α_2 and β -adrenoreceptors. Currently three α_1 receptor subtypes (α_{1A} , α_{1B} and α_{1D}), four α_2 receptor subtypes (α_{2A} , α_{2B} , α_{2C} and α_{2D}) and three β receptor subtypes (β_1 , β_2 and β_3) are recognized. All adrenergic receptors are coupled to different kind of G proteins (G_s , $G_{i/o}$, G_q).

2.3.3.1. α_1 -adrenergic Receptors

While it has been identified in the brain regions where all other adrenoreceptors are present such as cerebral cortex, it is also exclusively present in some brain areas including DRN, hippocampus²¹¹. Furthermore, it has been identified in the thalamus, amygdala, spinal cord and cerebellum²¹². α_1 receptors are usually coupled to the stimulation of membrane phospholipase C, leading to the formation of second messengers' inositoltriphosphate and diacylglycerol through G_q protein. Activation of the α_1 -adrenoreceptors decreases the K^+ conductance, thus making the neuron more

excitable. They are thought to exist at postsynaptic sites. In anesthetized rats, microiontophoretic and systemic administration of α_1 adrenoceptors antagonists suppress 5-HT neuron firing activity, which suggests a tonic facilitatory role for NA on the 5-HT activity. At the behavioural level these receptors participate to several pathologies such as depression, learning-memory deficiency (Table 5).

2.3.3.2. α_2 -adrenergic Receptors

α_2 -adrenoceptors are demonstrated in the LC, the thalamus²¹³ and few in the basal ganglion²¹⁴. It has been reported that the densities of α_2 adrenoceptors were higher in the LC and nucleus solitarius when compared to α_1 receptors. They are coupled to Gi/cAMP systems. α_2 receptors existing both pre and post synaptically. The main function of α_2 adrenergic receptors is the presynaptic regulation of transmitter release, where these receptors have been implicated in the inhibitory control of adrenergic and serotonergic pathways innervating the frontal cortex²¹⁵. Furthermore, the activation of α_2 receptors decreases NA output and suppresses the firing activity of 5-HT neuron of rat's DRN²¹⁶. Indeed, blockade of α_2 -adrenoceptors markedly enhances the increase of extracellular NA induced by NRIs²¹⁷ (Table 5).

2.3.3.3. β -adrenergic Receptors

Beta-adrenoceptors are predominantly located in the cerebral cortex, nucleus accumbens and striatum. At lower densities, they are also present in amygdala, hippocampus and cerebellum. Three β -adrenergic receptor subtypes have been cloned, β_1 , β_2 , and β_3 . Two subtypes, β_1 and β_2 , are more similar to each other than they are to the β_3 receptor.

β -adrenoceptors are 7 transmembrane receptors, which are all coupled to stimulation of adenylyl cyclase activity through G_s protein leading to the formation of the second messenger c-AMP²¹⁸.

It has been reported that they have a role in the mood, locomotion, analgesia, learning and memory. For example, Cartford et al.²¹⁹ showed that loss of cerebellar β -adrenergic receptor function correlates with a loss of function in related learning tasks.

Furthermore, as we detailed below the density of β -adrenoreceptors are changed by stress and during treatment of patients with antidepressants (Table 5).

Table 5. The properties of adrenergic receptors

Receptor	Anatomic	Agonist	Antagonist	Functions
α_1	Cerebral cortex, DRN, Hippocampus, Thalamus, Amygdala, Spinal cord, Cerebellum	Phenylephrine Methoxamine Oxymetazoline	Prazosin Doxazosin Tamsulosin	Locomotor activity Arousal, Vigilance Attention, Depression Memory
α_2	LC, basal ganglion, thalamus	Clonidine Clenbuterol	Yohimbine Idazoxan	Depression, Arousal Locomotion
β_1	Hippocampus, Cerebellum Hypothalamus	Denopamine RO363	Metoprolol (β_1) Atenolol (β_1)	Depression, Arousal Locomotion, Memory Analgesia, Learning
β_2	Hippocampus Cortex	Terbutaline Salmeterol	ICI 118,551 (β_2)	Depression
β_3	Cortex Hippocampus	BRL37344 SB251023	SR59230A	Depression Obesity

2.3.4. Noradrenaline and Mood Disorders

It is well established from a physiological point of view, that NA is involved in a whole range of processes, which are extremely important in the area of psychiatry, including learning and memory, sleep, arousal and adaptation. These actions are particularly relevant to depression, and also to the autonomic regulation and central manifestations of the stress response. Thus, NA is involved in a large number of brain functions, particularly during stress and major psychiatric illness.

2.3.4.1. Biochemistry of Noradrenaline and Depression

Evidence that NA plays a role in depression comes from a wide variety of sources, ranging from pharmacologic observations that specific drugs that either cause or improve depression appeared to affect NA turnover, to a number of biological observations in depressed patients. For example, reserpine depletes NA and causes symptoms of depression, whilst the TC and MAOI antidepressants increase the amount of available NA by blocking NA reuptake and degradation, respectively. Further support for this assumption come from the observations that α -MPT that inhibits the synthesis of the catecholamines produced a robust increase in the depressive symptoms on the Hamilton depression rating scale, in depressed patients in remission maintained by desipramine.

Measurement of MHPG in body fluids has been most widely applied to studies of depression and in attempts to predict the likelihood of a therapeutic response to antidepressant drugs. Some depressed patients have reduced levels of MHPG, the predominant metabolite of NA, in their urine and plasma and this subgroup appears to be responsive to treatment with NRIs. Although Maas et al.²²⁰ reported that approximately 60% of plasma MHPG is derived from the brain, it is currently believed that about one-third of plasma MHPG is of brain origin²²¹. So, it must be mentioned that the use of MHPG as indicators of activities in central noradrenergic neurons should be done with caution because it is predominantly derived from the periphery; however, plasma levels of MHPG may still give us valuable information regarding central noradrenergic activity²²².

2.3.4.2. The Noradrenaline Receptors and Depression

A reduction in the release of NA, possibly due to a decrease in neurotransmitter synthesis or an increase in (inhibitory) α_2 -autoreceptor activity, was one of the first hypotheses regarding a role for NA in major depression.

In animal studies, chronic stress induce number of changes in the noradrenergic system such as increased density of α_1 - and α_2 adrenoreceptors and reduced cyclic AMP response¹⁵.

Changes in α_2 -adrenergic receptor sensitivity after chronic therapy with antidepressants have been reported as well. The inhibitory α_2 adrenergic heteroreceptors are believed to control 5-HT release from the serotonergic nerve terminal²¹⁵, while α_2 adrenergic autoreceptors control the release of NA from noradrenergic nerve terminal, where blocking of these receptors will increase the release of NA from noradrenergic nerve terminals²²³. Sacchetti et al.²²⁴ suggested that the repeated treatment with desipramine facilitates its effect on extracellular NA in the dorsal hippocampus and induces desensitization of α_2 -adrenoreceptors with no changes in their density in the LC.

Likewise, the number and the function of postsynaptic α_1 receptors in the rat cortex were reported to be increased by the chronic administration of most antidepressants²²⁵.

Up-regulation of β -adrenergic receptors has been found consistently in patients with depression and their down-regulation is regarded as a marker for antidepressant activity¹⁶. Indeed, it was reported that the β -adrenergic receptor number and function are decreased in the rat cortex by the chronic administration (14 days) of desipramine, ECT²²⁶ or reboxetine²²⁷. Furthermore, Papp et al.¹⁷ showed that CMS elicits up regulation of β -adrenoreceptors without changing their affinity; it also increased the cyclic AMP response to NA in cortical slices. In addition, a strong tendency to increase the number of β -adrenoreceptors in the hippocampus after CMS has been reported²²⁸.

Moreover, the densities of β_1 adrenoreceptors are changed by stress, in several mood disorders including depression excessive hostility, schizophrenia and during treatment of patients with antidepressants²²⁹. In vivo and in vitro binding studies show that chronic treatment with antidepressant drugs and ECT down regulate the β_1 -adrenergic receptors in the rat forebrain²²⁶. In contrast, up-regulation β_1 -adrenoreceptors in the rat brain has been reported after chronic treatment with several SSRIs²³⁰. Other findings that support the role of NA in depression include an increased density of β_2 receptors in the brains of some suicide victims and the fact that blockade of NA synthesis results in relapse in depressed patients receiving noradrenergic treatments¹⁵⁰.

2.4. Tramadol

2.4.1. History of Tramadol

Tramadol [(1R,2R)-2-[(dimethylaminomethyl)-1-(3-methoxyphenyl)-cyclohexanol] is a synthetic aminocyclohexanol central analgesic belonging to the second step of the WHO scale which, unlike conventional opioids, exerts its analgesic activity through a dual mechanism of action, resulting in the inhibition of perception and pain transmission. The drug was developed in Germany by Grunenthal in 1962 and entered the market in West Germany in 1977, in the USA in 1995 and in the UK in 1997.

2.4.2. Pharmacology of Tramadol

Tramadol possesses a low affinity for opioid receptors with K_i values from 2.1 to 56.7 $\mu\text{mol/l}$ (Table 6) and lack of selectivity to the μ , κ and δ . The affinity of tramadol for the μ receptors is about 10, 60 and 6000 times weaker than that of codeine, dextropropoxyphene, morphine, respectively. It has been shown that tramadol enhances the extraneuronal concentrations of the monoamine neurotransmitters, NA and 5-HT, by interfering with the reuptake and release mechanisms^{3,4}.

Unlike other opioid receptor agonists, tramadol is a racemic mixture consisting of 2 enantiomers exerting different pharmacologic actions. The main activity of enantiomer (-) is the inhibition of NA reuptake, while (+) both interacts with μ opioid receptors and increases 5-HT synaptic concentrations via a mechanism similar to the one occurring at the noradrenergic level²³¹.

The overall activity of tramadol originates from the sum of specific actions of enantiomers and of its metabolite M1. This latter is characterized by its greater affinity with the μ receptor; it is thus the main responsible for opiate activity.

While the opioidergic system is the first mode of action of tramadol, the second one is its influence on the descending pain inhibitory system which include both descending, serotonergic and noradrenergic pathway⁴. So, actions other than opioid mechanisms may therefore contribute to its analgesic efficacy. This assumption is supported by the observation that in some experiments, effects of tramadol are not fully antagonized by naloxone²³².

Table 6. Affinity among tramadol, 2 enantiomers and the active metabolite M1 and opiate receptors and inhibition of 5-HT and NA re-uptake²³³. Note: The lower the Ki value the higher the affinity.

Product	Affinity with opiate receptors (K_i $\mu\text{mol/L}$)			Re-uptake inhibition	
	μ	δ	κ	NA	5-HT
(\pm)Tramadol	2.1	57.6	42.7	0.78	0.9
(+)Tramadol	1.3	62.4	54.0	2.51	0.53
(-)Tramadol	24.8	213	53.5	0.43	2.53
(+) M ₁	0.0034				
Morphine	0.00034	0.092	0.57	Inactive	Inactive
Imipramine	3.7	12.7	1.8	0.0066	0.021

Ki: constant of inhibition

2.4.3. Pharmacokinetics and Metabolism of Tramadol

Tramadol can be administered orally, subcutaneously, intravenously, intramuscularly, intraperitoneally or rectally. After oral administration, tramadol is rapidly absorbed, reaching its peak blood level after approximately 2h. Its elimination half-life is slightly above 6h.

Tramadol has a low plasma protein binding (about 20%) and it is widely metabolized in the liver by the cytochrome P-450 enzyme system²³⁴ and excreted by the kidney²³⁵.

Tramadol undergoes biotransformation in the liver, first by phase I reactions (mainly O- and N-demethylation), and second by phase II reactions (mainly conjugation of O- and N-demethylated compounds)²³⁶. In the first and second phase reactions 11 and 12 metabolites, respectively, are produced; the main metabolite is O-desmethyltramadol (M1)²³⁷.

2.4.4. Tramadol and Analgesia

Tramadol is a centrally acting analgesic for the prevention and treatment of moderate to severe pain in acute or chronic conditions. Tramadol interacts at different levels of the nociceptive system in both the supraspinal and spinal areas²³³.

It is effective in a whole range of pain including intra-operative, post-operative, musculoskeletal, neuropathic²³³ and cancer pain²³⁵.

2.4.5. Tramadol and Depression

Since both monoaminergic and opioid systems have been implicated in depressive disorders, tramadol has been studied in the FST in mice⁶ and learned helplessness model⁷. There are also some clinical studies which have also shown that tramadol has been used in some psychiatric situations such as refractory major depression⁹, suicidal ideation²³⁸ and obsessive-compulsive disorder⁸.

The study of Rojas-Corrales et al.⁶ have shown that, indeed, tramadol displays antidepressant-like effect in mice, mediated by the noradrenergic system, rather than the serotonergic or opioidergic system. In line with evidence that tramadol displays an antidepressant action, another study was undertaken to examine the neurochemical effects of long-term tramadol administration. This study revealed that specific frontocortical [³H] dihydroalprenolol (β -adrenergic receptor antagonist) and [³H] ketanserin (serotonin 5-HT_{2A} receptor antagonist) binding was lower in the group receiving tramadol for 21 days, as compared to the control group²³⁹, indicating the similarity in the effects evoked by repeated administration of tramadol and by the majority of conventioned antidepressants. In addition, Faron-Gorecka et al.²⁴⁰ reported that tramadol induced a downregulation of α_2 -adrenergic receptors that could result in a significant increase in the amount of 5-HT in the synaptic cleft. So, this can be a evidence for the possible antidepressant-like effect of tramadol, when compared with the action of already ongoing antidepressants. Indeed, the interaction with the monoamine neurotransmission of (+)-enantiomer, (-)-enantiomer or (\pm) tramadol closely resembles that of antidepressant drugs, being either SSRIs, NARI or both SNRIs, respectively.

On the other hand, tramadol has structural (Fig.10) and pharmacological similarities with the antidepressant venlafaxine who is also racemic mixture and has effects on the reuptake of the NA and 5-HT like tramadol⁵.

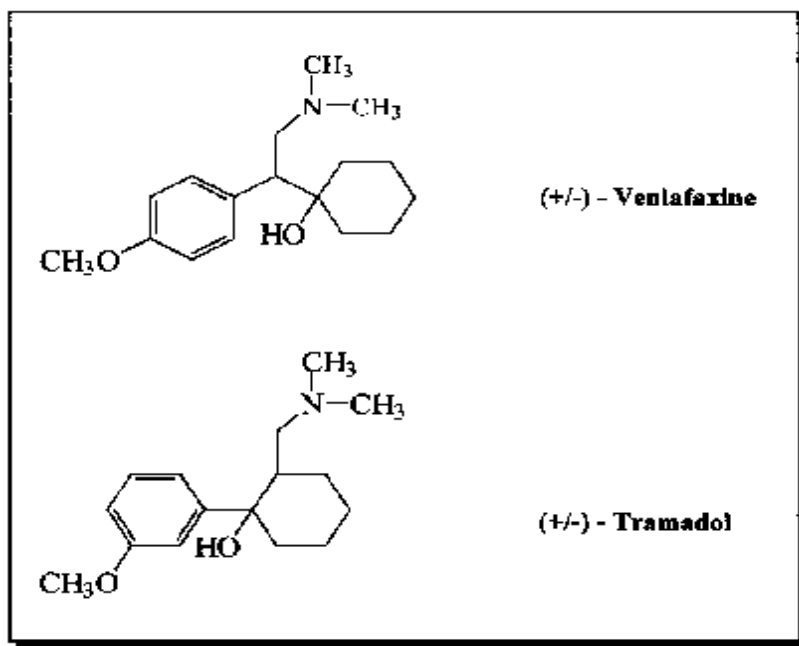


Fig.10. Chemical structure of venlafaxine and tramadol

2.4.6. Tolerability and Therapeutic Safety of Tramadol

Tramadol has been widely investigated also for its profile of tolerability and therapeutic safety^{241, 242}.

The gastrointestinal symptoms, such as nausea and vomiting, are relatively frequent, especially at the beginning of the treatment²⁴³.

Several long-term clinical studies have proven that the frequency of constipation and nausea is lower with tramadol than morphine²⁴⁴.

Versus the traditional non-opioid and opioid analgesics, tramadol offers some advantages, because it does not cause organ damages and does not induce severe side effects. It is worth to remark that tramadol, at therapeutic doses, exerts poor or null

effects on the respiratory, immune, cardiovascular system and on intestinal motility^{234, 245, 246}. Tramadol exerts no immunodepressive activity either in animals or in humans. Therefore, it can be used in patients whom the immunosuppressive action is contraindicated such as elderly, diabetic patients, patients with reduced immune defenses due to specific disease and /or therapies.

2.4.7. Abuse and Dependence

A recent experimental study performed in rats has proven that tramadol, other than morphine, poorly interacts with the endorphin system to induce dependence²³³. In human study, it has been reported that it makes very low potential of abuse.

In the United States, tramadol has been sold since 1995 and it is freely prescribed owing to its low potential abuse, whereas in Turkey the specialist prescribes via the green receipt and in France it is given to patients under control.

2.5. Desipramine

2.5.1. Pharmacology of Desipramine

Desipramine displays antidepressant properties similar to that of other tricyclic antidepressants. It is the active "in vivo" metabolite of imipramine and as such, shares many of imipramine's pharmacologic effects. A difference with the imipramine that it has a selectivity for NA. It is well known that desipramine exerts in vivo an inhibitory effect on NA reuptake in the LC and cingulate cortex, thus the result of the blockade of NA reuptake is a sustained increase in extracellular levels of NA in both areas. Furthermore, it has been reported that the effect of desipramine on extracellular NA in the brain cortex is modulated by α_2 -adrenoreceptors in the LC²⁴⁷.

2.5.2. Pharmacokinetics and Metabolism of Desipramine

Desipramine is easily absorbed from the gastrointestinal tract following oral administration and is extensively bound to tissue and plasma proteins in the order of 90 to 95%. It is inactivated by hydroxylation and by further demethylation in the liver.

Desipramine is excreted as a glucuronide largely in the urine (approximately 70%) and partly in the bile.

2.5.3. Desipramine and Mood Disorders

It is effective in major depression as well as bipolar disorders and anxiety. Like other antidepressants, its effect onset after the 2-3 weeks treatments. In preclinical studies, antidepressant-like effects have been reported in the FST²⁴⁸, learned helplessness model²⁴⁹ and TST²⁵⁰. In addition, desipramine was introduced in an attempt to reverse or prevent stress-induced behavioural deficits and help evaluate the predictive validity of CMS model²⁵¹. Because of its efficacy in the various animal models and in clinic, it is also useful tool to compare the effects of novel antidepressant drugs.

2.5.4. Tolerability and Therapeutic Safety of Desipramine

The more common adverse reactions involve anticholinergic effects such as dry mouth, disturbances of visual accommodation, constipation and mild urinary retention. Also commonly seen are light headedness, drowsiness, increased perspiration and mild tremors as well as insomnia. Adverse reactions of the cardiovascular system may be much more serious, however, these occur less frequently such as hypotension, hypertension, tachycardia, palpitation, arrhythmias^{252, 253}.

3. MATERIAL and METHODS

3.1. Animals and Accommodation

Male, inbred BALB/c ByJ and Swiss mice were used for the experiments. The mice were obtained from the Department of Physiology, University of Cukurova, Adana and the Centre d'Elevage Janvier for the experiments, which were realized in Turkey and France, respectively. The experiments were carried out with 8-9 weeks old mice, which were housed five per cage in transparent cages including 1.5 cm sawdust. Animals were kept in the laboratory for 2 weeks before the onset of the experiments. During this period and along the experiments, the animals received food and water ad libitum.

Along the stress procedure, all stressed mice were maintained individually under the same conditions while non-stressed mice were housed five per cage and kept in regulated environment 24 ± 1 °C, 12h/12h light/dark cycle (lights on at 20:00 and off at 8:00). The dimensions of the home cages for the stressed mice are 14 cm height, 29 cm long and 17 cm large, for the non-stressed mice 19 cm height, 39 cm long and 24 cm large. During the stress procedure the stressed mice and non-stressed mice were accommodated separately in different rooms.

The Swiss mice were only used for the experiments that we aimed at searching the strain differences. For the rest of the experiments BALB/c ByJ strain was used.

This thesis was conducted in accordance with the European Community guidelines for the use of experimental animals.

3.2. The Unpredictable Chronic Mild Stress Model (UCMS)

The UCMS regimen used in this study was based on the procedure originally designed by Willner et al.⁹⁹ for rats and adapted for mice by Kopp et al. and Ducottet et al.^{41, 254}. Mice were subjected several times a day for 6 weeks to one of the mild stressors that we detailed in Table 7 in a chronic, inevitable and unpredictable way. To prevent habituation and to provide an unpredictable feature to the stressors, all the stressors and/or sequence were administered at different time points every week. For

ethical reason, the stress procedure did not involve food and water deprivation or immobilization.

Except the social stress and cage changing, the mice were exposed to the stressors in their own cage. The stressors were;

- cage tilt at 45°.
- damp sawdust (soiled cage) (100 ml of water spilled onto the bedding)
- switching cages. There are two types of switching cages. If one of the mice was put in the cage of the other mice and stayed there, we named it social stress. After a period of time if we put it again in its own cage, this stressor was called cage changing. For these stressors, the odour of the other animals is carry as aversive stimulus.
- inversion of the light/dark cycle.
- lights on for a short time during the dark phase or inverse.
- sounds of predators during 15 minutes
- placement in an empty cage realized by evacuating the sawdust which is in its own cage
- bath was made by the placement in an empty cage of 250 ml water on the bottom

Table 7. Procedure of the UCMS model. The hours, which are in the paranthesis, show the duration of stressors.

Weeks	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1	coat state, weighing (10h) social stress (12h)	social stress (9h)	3 sawdust changing (10h30-11h30)	social stress (12h)	damp sawdust (9h30-11h)	reversal of the light/dark cycle (after 8h)	reversal of the light/dark cycle
	without sawdust (15h-17h)	sounds of predators (15h)	social stress (15h)	cages tilt at 45° (14h-15h30)	Social stress (14h)	reversal of the light/dark cycle	reversal of the light/dark cycle
Week 2	end of reversal cycle (8h) coat state, weighing (10h)	without sawdust (9h30-12h30) + social stress (11h)	damp sawdust (10h-13h)	social stress (12h)	dark (5h-6h) 2 sawdust changing (10h-11h)	4 light/dark succession every 30 min. (9h30-11h30)	4 light/dark succession every 30 min. (9h30-11h30)

Table 7. (Continue) Procedure of the UCMS model. The hours, which are in the paranthesis, show the duration of stressors.

Weeks	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 2	sounds of predators (13h) cages tilt at 45° (14h-15h30)	cages tilt at 45° (15h-16h30)	social stress (18h)	sounds of predators (16h) light (19h-20h)	Social stress (15h)	light (15h-17h) dark (4h-6h)	light (15h-17h) dark (4h-6h)
Week 3	coat state, weighing (10h)	cages tilt at 45° (11h-14h)	damp sawdust (8h30-11h30) + social stress (10h)	sounds of predators (10h)	light (9h-9h30) sawdust changing (10h)	reversal of the light/dark cycle	reversal of the light/dark cycle
	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)
	without sawdust (15h-16h30)	light (17h-18h)	cage changing (14h30-17h30)	bath (15min) (15h)	without sawdust (14h30-17h30) Reversal of the light/dark cycle (after 20h)	reversal of the light/dark cycle	reversal of the light/dark cycle. End of reversal cycle (after 20h)
Week 4	coat state, weighing (10h)	sounds of predators (11h)	cage changing (9h-12h)	cage changing (9h-9h30)	sawdust changing (10h)	light (9h-11h)	end of reversal cycle (after 8h)
	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)
	cages tilt at 45° (14h30-15h30) cages tilt at 45° (17h-18h)	damp sawdust (15h-18h) + social stress (16h30)	lights (30min) (15h-16h30-18h)	damp sawdust (14h30-17h30)	bath (15min) (14h30)	reversal of the light/dark cycle (after 20h)	light 30min (15h15-16h15-17h15-18h15-19h15)
Week 5	coat state, weighing (10h)	cages tilt at 45° (10h-13h)	stress social (11h)	sounds of predators (10h30)	dark (5h-6h) bath (15min) (10h)	4 light/dark succession every 30 min. (9h30-11h30)	dark (4h-6h) 4 light/dark succession every 30 min. (9h30-11h30)
	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)
	without sawdust (16h-17h30)	social stress (15h) light 30min (17h-18h-19h)	damp sawdust (14h30-17h30)	without sawdust (14h30-17h30) + social stress (16h-17h30)	2 sawdust changing (15h-16h)	light (15h-17h)	light (15h-17h)
Week 6	dark (4h-6h), coat state, weighing (10h)	behavioural tests	behavioural tests	behavioural tests	behavioural tests	behavioural tests	behavioural tests
	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)	treatment (13h30)

3.3. Experimental Protocol

For all the experiments, the mice were subjected to the UCMS regimen. At the end of 2 weeks of a drug-free stress exposure, mice were assigned to the different experimental groups in a semi-randomized manner, so that the initial coat state and body weights were equivalent in all the groups. All the non-stressed groups also received the same dose of drug administration at the same moment. Four main experiments were performed in this study:

In the first experiment, the effects of strain differences and different types of antidepressants were searched in the UCMS.

In the second experiment, the effects of tramadol and desipramine on the UCMS model in BALB/c mice were studied

In the third experiment, the contribution of the serotonergic system to the antidepressant-like effects of tramadol and desipramine in the UCMS in BALB/c mice was studied.

In the fourth experiment, the participation of the noradrenergic system in the antidepressant-like effects of tramadol and desipramine in the UCMS in BALB/c mice was examined.

3.3.1. The procedure for the Effects of Strain Differences.

To investigate the potential contribution of the genetic variation to the UCMS model, one outbred (Swiss) and one inbred (BALB/c) mice groups were used. The reason for choosing these strains is that they are the most frequently used strains in psychopharmacological tests¹². In order to examine the drug action, we choose four antidepressants, which show their effects via different mechanisms: two TCAs (imipramine, a mixed serotonergic noradrenergic reuptake inhibitor and desipramine, a specific noradrenergic reuptake inhibitor), one tetracyclic antidepressant (maprotiline which strongly inhibits the uptake of NA, though it is notable in its lack of inhibition of serotonergic uptake) and SSRI (fluoxetine). In order to determine the effects of the UCMS regimen and drug treatment, two parameters were used: the coat state and the splash test. The state of the coat was used to evaluate grooming behaviour indirectly whereas the splash test determined this behaviour directly³³.

3.3.2. The Procedure Assessing the Effects of Tramadol and Desipramine in the UCMS Model in BALB/c Mice.

According to the results obtained from the first experiments, the BALB/c strain was selected for the rest of the study. In this part, we studied the effects of tramadol and desipramine in stressed and non-stressed BALB/c mice. The state of the coat of mice and the splash test was used to interpret the grooming behaviour. The actograph was used to evaluate the locomotor activity during four hours. Morris water maze test was also realized. These behavioural tests were detailed below.

As tramadol is able to bind to μ receptors, naltrexone, an opioid receptor antagonist, was used in order to search the participation of the opioidergic system in the antidepressant-like effects of tramadol and desipramine. However, as the mixture of tramadol and naltrexone augmented the mortality of mice, we could not achieve to finish the stress procedure.

3.3.3. The Procedure for the Contribution of the Serotonergic System to the Antidepressant-like effects of Tramadol and Desipramine in UCMS Model in BALB/c mice.

In this part of the study, the effects of a 5, 7-DHT lesion of the DRN on the antidepressant-like effects of tramadol and desipramine were examined. In addition, the participation of the 5-HT_{1A} receptor which is the most extensively studied among the 5-HT receptors was examined via 5-HT_{1A/1B} receptor antagonist pindolol in the antidepressant-like effects of tramadol and desipramine.

3.3.3.1. The Effect of a 5, 7-Dihydroxytryptamine (5, 7-DHT) Lesion on the Antidepressant-like Effects of Desipramine and Tramadol in the UCMS Model

3.3.3.1.1. 5, 7 DHT and Surgery

The depletion of 5-HT neurotransmission in brain by neurotoxins is a method commonly used in particular to investigate the mechanism of action of drugs or the effects of stress in the psychopharmacological studies. For this purpose, parachlorophenylalanine (PCPA) methyl ester hydrochloride or 5, 7-DHT is frequently used

but their mechanism of action is unlike. PCPA is a tryptophan hydroxylase inhibitor²⁵⁵, hence it decreases the synthesis of 5-HT. On the other hand, 5, 7-DHT is an analog of 5-HT that destroys 5-HT axons and terminals when injected into the brain²⁵⁶. The other important difference is that SSRIs (fluoxetine, chlorimipramine) do not block the ability of 5, 7-DHT to deplete 5-HT pools and slow 5-HT synthesis rate in the rat brain even if they are administered at high doses. In contrary, 5-HT reuptake blocker fluoxetine did block the 5-HT depletion that occurs following an injection of PCPA. In addition 5, 7-DHT also reduces the concentrations of 5-HIAA. But it is not completely specific for 5-HT neurons, it can also destroys NA neurons²⁵⁶.

For the surgery, the mice were divided into two groups; while one group was lesioned with the neurotoxin 5, 7-DHT, the other group received 0.2 % ascorbic acid, the vehicle of the neurotoxin (sham). Twenty minutes before the infusion of the 5, 7-DHT or its vehicle into the DRN, the mice were pretreated with NRI, desipramine (25 mg/kg, i.p), to prevent destruction of noradrenergic terminals by the neurotoxin²⁵⁷. The animals were then anesthetized intraperitoneally (i.p) with the mix of ketamine (80 mg/kg) and xylazine 2% (14 mg/kg). This type of anesthesia provides a good sedation, analgesia and myorelaxation. It also induces less hypothermia and the duration of anesthesia is short compared to other anesthetic such as pentobarbital.

The mice were then fixed in the stereotaxic apparatus with 2 ear bar and an incisor bar (Fig.11). After that, we made a cutaneous incision on the surface of the skull. The stereotaxic coordinates for the DRN were AP= -4.4mm; Lat=0 mm; V= +3.1 mm relative to bregma, AP=+0.5 mm; Lat=0mm; V=+3.1 mm relative to lambda. (AP: Anteroposterior, Lat: Lateral, V: Vertical from the skull surface). On this point, a hole was made by the drill in order to introduce the cannula. The injection of the vehicle (0.2 % ascorbic acid) (sham) or 5, 7-DHT (1µg/0,2µl) (lesion) was made after the cannula was lowered into position and infused in a volume of 0,3 µl over a period of 3 min by a Microinfusion pump (Diameter: 0,15 mm, rate: 0,1 µl/min). The cannula was removed 2 min after the completion of the infusion and the incision sutured thereafter.



Fig.11. Fixation of the mouse on the stereotaxic apparatus.

Before the beginning of the real experiment, preliminary essays were realized to be sure of the placement of the infusion. For this purpose, 0, 25 μ l methylene blue was administered into the DRN. Ten minutes after, the mice were decapitated by cervical dislocation and the brain was taken out and was kept at -20°C until use. The verification of the localization of the methylene blue was realized by the visualization of the blue color on the slice of the brain (Fig.12).

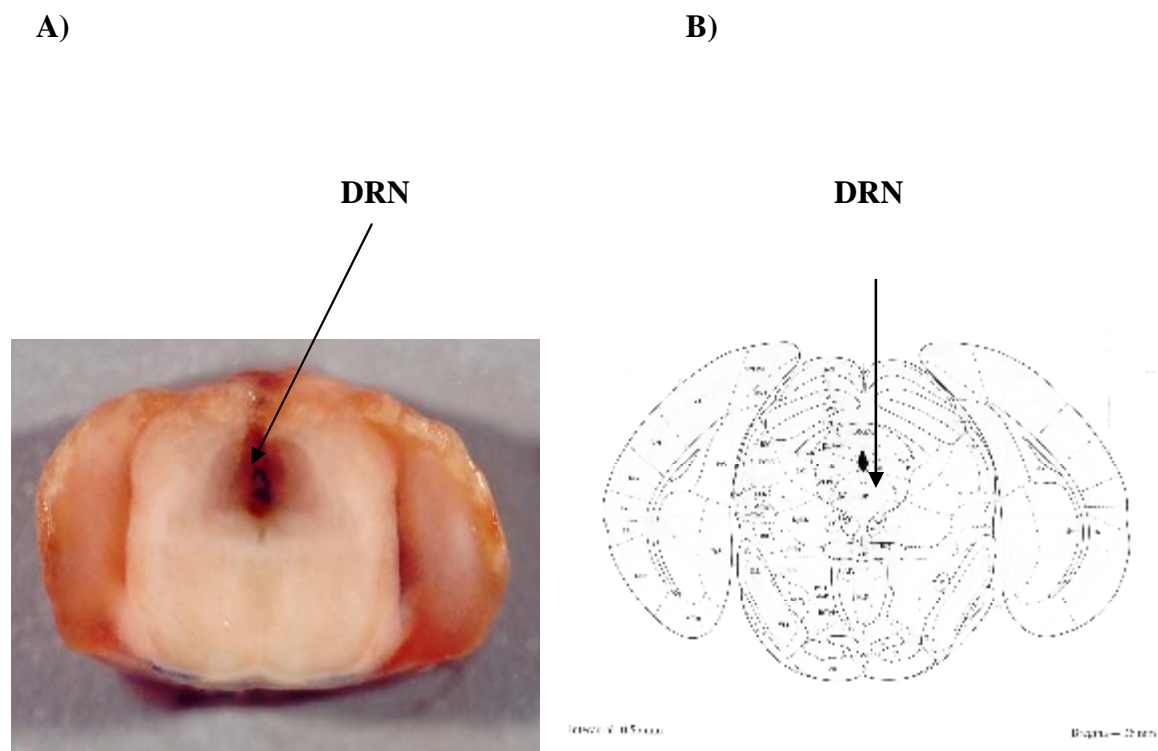


Fig.12. A) Frontal slice of a mouse brain showing the DRN colored by the methylene blue. B) The figure from the atlas of the mouse brain in stereotaxic coordinates²⁵⁸ shows a frontal slice of the mouse brain as same level as FigA.

3.3.3.1.2. Stress Procedure

After 1-1.5 week of post-operative recovery period, the mice were divided into non-stressed and stressed group in a semi-randomized manner. The mice were then subjected to the UCMS regimen as detailed before. To evaluate the effects of the lesion or the drug treatments, the state of the coat, the changes in the body weights, the locomotor activity and the resident-intruder test were realized. The locomotor activity was recorded during 2h.

3.3.3.1.3. Biochemistry

Once the behavioural tests were completed, forty eight hour after the last injection, the mice were killed by rapid cervical dislocation. The brain was rapidly removed from the cranium and dissected on ice. The hippocampus, striatum, frontal cortex, cerebellum and the region of raphe magnus were dissected and weighed into propylene tubes.

Each section was homogenized in 1 ml of a buffer containing 12 M HClO₄, 0.1 M EDTA, 0.5 mM Na₂S₂O₅, 3 mM octanesulfonic acid and 3 mM heptanesulfonic acid with an Ultraturrax T25 at 4°C. After a 30.000xg centrifugation at 4°C for 20 min, 100 µl of the supernatant was kept at -80°C until use. The levels of NA, DA, 3,4 dihydroxyphenylacetic acid (DOPAC), HVA, 5-HIAA and 5-HT were measured in each supernatant by HPLC with electrochemical detection (ED) on a Concorde apparatus (Waters, St.Quentin-Yvelines, France). The mobile phase consisting of 10.5 % acetonitril, 4.5% methanol and 85% 20 mM citric acid, 10mM monobasic phosphate sodium, 3 mM octasulfonic acid, 3.25 mM heptanesulfonic acid, 0.1 mM EDTA, 2 mM KCl, 6 ml/l o-phosphoric acid and 2 ml/l diethylamine with ph 3± 0.1 was pumped at 0.3 ml/min with a Gold 118 system (Beckman, Fullerton, CA). Separation was performed with a 3-µm C18, 3.2x 100 mm reversed phase column (LC-22C, BAS, West Lafayette, IN). A glassy carbon working electrode set at 610 mV with reference to an in situ Ag/AgCl reference electrode was used to detect compounds. Signals were recorded and quantified with a Beckman Gold 118 integrator. For each analysis, a set of standards containing 50, 100 and 200 nM of each compound was prepared in the acid solution and the calibration curves were calculated. The concentrations of compounds in the supernatant were determined from the peak area of each compound and compared with the standard curve of 50,100 and 200 nM of standard solution. All data are expressed in terms of µM/g fresh tissue.

3.3.3.2. The Effects of Pindolol on the Antidepressant-like Effects of Tramadol and Desipramine on the UCMS Model in BALB/c Mice.

As no effects of the pharmacological treatments were observed in non-stressed mice in the first and second experiments, we did not include these non-stressed groups

in this experiment, which aimed at investigating the participation of 5-HT_{1A/1B} receptors in the antidepressant-like effects of desipramine and tramadol. For this purpose, the 5-HT_{1A/1B} receptor antagonist pindolol was tested alone or in combination with either tramadol or desipramine.

This study consisted of 6 groups; Vehicle + vehicle, tramadol + vehicle, desipramine + vehicle, pindolol+vehicle, tramadol+pindolol, desipramine+pindolol. The state of the coat and body weight of mice was examined every week. The grooming behaviour was evaluated by the splash test on the sixth week of the stress procedure. The actograph was also realized at the beginning of the sixth weeks.

3.3.4. The Procedure for the Participation of the Noradrenergic System in the Antidepressant-like Effects of Tramadol and Desipramine on the UCMS Model in BALB/c Mice

3.3.4.1. Determination of NA and its Metabolite MHPG Level After the End of the UCMS Model: the Effects of Tramadol and Desipramine

In this part of the study, the effects of tramadol and desipramine on the brain NA and MHPG level were examined. For this purpose, the mice were submitted to an UCMS regimen during 6 weeks. After the 48 hours of the last injection, the mice were killed by rapid cervical dislocation. The brain was rapidly removed from the skull and dissected on ice. The brain samples such as the region of the LC and hypothalamus, hippocampus and cerebellum were dissected and weighed into polypropylene tubes. Every sample was homogenized in 1 ml of a buffer containing 12 M HClO₄, 0.1 M EDTA, 0.5 mM Na₂S₂O₅, 3 mM octanesulfonic acid and 3 mM heptanesulfonic acid with an Ultraturrax T25 at 4°C. After a 30.000xg centrifugation at 4°C for 20 min, 100 µl of the supernatant was kept at -80°C until use. The levels of MHPG and NA were measured in each supernatant by HPLC-ED on a Concorde apparatus (Waters, St.Quentin-Yvelines, France). The mobile phase consisting of 3.5% acetonitril, 1.5% methanol and 95% 20 mM citric acid, 10mM monobasic phosphate sodium, 3 mM octasulfonic acid, 3.25 mM heptanosulfonic acid, 0.1 mM EDTA, 2 mM KCl, 6 ml/l o-phosphoric acid and 2 ml/l diethylamine with pH 3± 0.1 was pumped at 0.3 ml/min with a Gold 118 system (Beckman, Fullerton, CA). Separation was performed with a 3-µm

C18, 3.2x 100 mm reversed phase column (LC-22C, BAS, West Lafayette, IN). A glassy carbon working electrode set at 610 mV with reference to an in situ Ag/AgCl reference electrode was used to detect compounds. Signals were recorded and quantified with a Beckman Gold 118 integrator. For each analysis, a set of standards containing various concentrations of each compound was prepared in the acid solution and the calibration curves were calculated. The concentrations of compounds in the supernatant were determined from the peak area of each compound and compared with the standard curve of 100 nM of standard solution. All data are expressed in terms of $\mu\text{M/g}$ fresh tissue.

3.3.4.2. The Effects of Propranolol, ICI 118,551 and Yohimbine on the Antidepressant-like Effects of Tramadol and Desipramine on the UCMS Model in BALB/c mice

In order to investigate the contribution of β , β_2 and α_2 -adrenoreceptors, tramadol and desipramine were combined with propranolol (non-selective β -adrenoreceptor antagonist) or ICI 118,551 (β_2 -adrenoreceptor antagonist) or yohimbine (α_2 -adrenoreceptor antagonist), respectively. This study included 3 main sections. In experiment 1, the effects of β -adrenoreceptors on the antidepressant-like effects of tramadol and desipramine were searched in the non-stressed and stressed mice. As no effects of the pharmacological treatments were observed in non-stressed mice in this group, non-stressed groups were not included in the second and third sections in which the effects of ICI 118,551 and yohimbine, respectively were searched.

Experiment 1 consisted of 12 subgroups. The subgroups were as follows: vehicle + vehicle, tramadol + vehicle, desipramine + vehicle, propranolol + vehicle, tramadol + propranolol, desipramine + propranolol in stressed and non-stressed mice.

Experiment 2 included 6 subgroups. They were as follows: vehicle+vehicle, tramadol + vehicle, desipramine + vehicle, ICI 118,551 + vehicle, tramadol + ICI 118,551, desipramine + ICI 118,551 in stressed mice.

Experiment 3 also included 6 subgroups: vehicle + vehicle, tramadol + vehicle, desipramine + vehicle, yohimbine + vehicle, tramadol + yohimbine, desipramine + yohimbine in stressed mice.

Before and during the UCMS regimen, the state of the coat was evaluated and on the sixth week of the UCMS regimen the splash test was realized. For the first and second experiments, the locomotor activity was recorded during 15 min. In experiment 3, the locomotor activity was recorded for 4h.

3.4. Drugs

3.4.1. The anesthetics

The drugs used for the anesthesia:

-**imalgéne 500** (Merial, France): It is a soluble injectable form of ketamine hydrochloride. Ketamine, the NMDA receptor antagonist that frequently use for the anesthetic effects in human and also in rodent studies.

Dose: 80 mg/kg

-**rompun 2%** (Bayer, France): It is an injectable form of xylazine hydrochloride. It is an agonist of α_2 -adrenereceptors, which has also analgesic and myorelaxant effects.

Dose: 14 mg/kg

All the anesthetics were diluted in saline (NaCl 0,9 %).

3.4.2. The Pharmacological Agents Tested

5, 7-DHT (Sigma, France): an analog of 5-HT that destroys 5-HT axons and terminals. To prevent oxidation of the neurotoxin, 5, 7-DHT was dissolved in a 0.2 % ascorbic acid/saline solution just prior to injection.

Dose: 1mg/0,2 ml

Tramadol: a centrally acting analgesic. It was supplied as a gift by Abdi Ibrahim (Istanbul, Turkey) and the Max Planck Institute of Psychiatry (Munich, Germany).

Dose: 20 mg/kg

Desipramine (Sigma): a TCA antidepressant, which strongly inhibits the reuptake of NA

Dose: 10 mg/kg

Imipramine (Sigma): a TCA antidepressant, which is a mixed serotonergic noradrenergic reuptake inhibitor.

Dose: 20 mg/kg

Maprotiline (Sigma): a tetracyclic antidepressant, which strongly inhibits the reuptake of NA, though it is notable in its lack of inhibition of serotonergic reuptake.

Dose: 5 mg/kg

Fluoxetine (Sigma): SSRI.

Dose: 10 mg/kg

Pindolol (Sigma): a 5-HT_{1A/1B} receptor antagonist. It has a weak antagonistic effect on the β -adrenoreceptors.

Dose: 10 mg/kg

Yohimbine (Sigma): a α_2 -adrenergic receptor antagonist.

Dose: 2 mg/kg

Propranolol (Sigma): a non-selective β -adrenergic receptor antagonist

Dose: 5 mg/kg

ICI 118,551: a selective β_2 -adrenergic receptor antagonist. It was kindly provided by Astra Zeneca (Cheshire, UK).

Dose: 2 mg/kg

Naltrexone (Sigma): an opioid receptor antagonist

Dose: 3 mg/kg

Unless noted, all the drugs were dissolved in saline solution and administered i.p. When mice were injected with two compounds, the drugs were mixed within one solution before the administration in order to avoid multiple injections. I.p injections were given daily at 1:30 p.m in a volume of 0.1 ml/10 g body weight. Vehicle groups received the same volume of 0.9 % NaCl.

3.4.3. The Products Used for the Biochemistry

MHPG, (\pm) Arterenol (NA), HVA, 3-hydroxytryamine (DA), DOPAC, 5-HT, 5-HIAA were supplied by Sigma. They were dissolved in 0,12 N perchloric acid.

3.5. The Behavioural Tests

3.5.1. The Evaluation of the State of the Coat and the Body Weight

The state of the coat and the body weight of the mice are important indicators of the general state. It has already been reported that the UCMS procedure induces a degradation of the general state which can be counteracted by a chronic antidepressant treatment but not by acute administration^{33,41}.

Before and during the UCMS, the state of the coat and body weight of the animals was recorded each monday. The evaluation of the coat state was carried out by the assessment of eight different body parts: head (including eyes and nose), neck, dorsal coat, ventral coat, tail, forepaws, hindpaws and genital regions^{41, 259}. A score of 0 for a coat in a good state or a score of 1 for a dirty coat were given for each of these areas. Dirty state is characterized by fluffy, greasy, less dense coat or piloerection. In addition, conjunctivitis and rhinitis were observed (Fig.13). Total score was obtained from the sum of the score of each body parts. If it's not indicated otherwise, in all the experiments, the total score of the last week of the stress regimen was presented. For example, in the experiment that we searched the antidepressant-like effects of tramadol and desipramine further more the experiment that we aimed at investigating possible effects of pindolol on the onset of the antidepressant-like action of desipramine and tramadol, the total score of each week was shown.

The observers who scored the state of the coat were unaware of the treatment condition.

Before UCMS



After UCMS



Fig.13. The state of the coat before and after the UCMS regimen in BALB/c mice.

3.5.2. Splash Test

The aim of this test is to determine the grooming behaviour. Grooming corresponds to the cleaning of the fur of the animal by licking or scratching. At the beginning of the sixth week, the splash test was performed. This test was realized for the grooming behaviour of both stressed and non-stressed mice. Non-stressed mice were isolated 5h before the test. Grooming bouts were recorded including nose/face grooming (strokes along the snout), head washing (semicircular movements over the top of the head and behind the ears), body grooming (body fur licking)²⁶⁰. An organizer (Psion Organizer Model LZ64, UK) was used to record the frequency of grooming, which refers to the number of licking during five minutes. For this purpose, a 10 % sucrose solution was squirted on the dorsal coat of mice in their homecage. The frequency and the duration of grooming were recorded during five minutes after the vaporisation of the sucrose solution²⁵⁹. We display the number of grooming behaviour as total frequency in the graphics.

The observer was unaware of the treatment conditions.

3.5.3. Actograph

The actograph was used to examine the spontaneous locomotor activity. This test was realized in two different manners. For the experiments which were performed in France, the procedure was as follows: the activity was recorded during 2 or 4 hours using a photo-electric actimeter²⁶¹. This procedure allowed evaluating the activity of mice in their homecage excluding the possible effects of new environment on the locomotor activity. The homecage was placed in the centre of the device, which consisted of a 20 cm x 20 cm square plane with two electrical eyes. The infrared beams were placed outside of the cage at a height of 2.8 cm, sufficient to detect the mice movements and elevated above the level of the sawdust in order to permit data recording. When the mice crossed throughout, the movement of the animal was detected automatically.

For the experiments which were conducted in Turkey such as the experiments aimed at searching the effects of propranolol and ICI 118,551, the locomotor activity was monitored with an electrical activity cage with a 24cm large, 37cm long, and 25cm heights. When the mice crossed throughout, the movement of the animal was detected automatically by the bars, which are 1 cm above the floor. To examine spontaneous locomotor activity, both non stressed and stressed mice receiving the drugs or vehicle were placed in the activity cage (Ugo Basile, 7400), and their activity (number of beam crossings) was evaluated for 15 min.

Non-stressed mice were isolated 18h-20h before the test.

3.5.4. Resident-Intruder Test

The resident-intruder test was carried out like previously described by Mineur et al.²⁶². Non-stressed mice were isolated 42h before the test, during which the bedding was not changed in order to increase the amount of territorial cues within cages. Mice were tested against a male A/J intruder, known for its high passivity and lack of aggression²⁶³. The opponent (intruder) was placed into the cage of the test animal (resident) in such a way that mice were placed in opposite corners. Then the test started immediately, lasting for a maximum of 10 min. Previous experiments showed that if an animal did not attack within this period, it was not likely to attack at all²⁶⁴. Tests were

stopped after the first attack occurred. We recorded the latencies of the first tail rattling and of the first attack.

3.5.5. Morris Water Maze

This test was used to evaluate the spatial memory and learning; it was developed by Morris et al.²⁶⁵. Mice were placed in a white-plastic circular pool (90 cm diameter and 30 cm height) filled with water (14 cm height, 25 ± 1 °C) made opaque by addition of plastic bead. The experimental room was lighted with halogen light (10 lx). The pool was subdivided in four equals imaginary quadrants called arbitrarily N, S, E and W. A platform (5cm x 5cm square plane) was placed 1 cm above or 1 cm below the water level in the centre of W according to the experimental conditions. Several extra-maze cues (posters and objects) could serve to locate the platform. This test consisted of three parts. The first part is a familiarisation with the apparatus and procedure. During this part, the platform is visible (1cm over the water) in the W part of the pool. Mice received three trials, each trial starting from a different point at the perimeter of the pool. For each trial (maximum 60 s), the time necessary to find the platform was recorded by an organiser. Animals had 60 s to rest on the platform between each trial. Mice failing to find the platform within 60 s were placed onto the platform and were allowed to remain on the platform for 60 s before beginning the next trial. The second part corresponds to learning: animals had to learn the location of a submerged platform placed in the centre of the W quadrant, in order to escape from water. This part included 4 sessions, each session being composed of 3 trials. The order of the departure was different for each session. The third part is the test session. After the last learning session, animals retention was tested over a 1-min probe trial. Mice were placed into the pool without the platform and the time spent in the W quadrant (the part in which the platform was previously located) was recorded.

3.6. Statistics

As data did not fit the conditions for using parametric test (homogeneity of variances or number of subject), non-parametric tests were used to interpret the results.

General comparison among groups was achieved by the Kruskal-Wallis ANOVA, if this test was significant ($P < 0.05$); Mann-Whitney U test was used to compare two groups. All the statistical analyses were carried out using the SPSS software 10.00. $P < 0.05$ was considered statistically significant. The results were expressed as the mean \pm S.E.M. Every group consisted of 8-12 subjects.

4. RESULTS

4.1. The Effects of Strain Differences and Validation of Some Antidepressants in the UCMS

In this part, the purpose was to find out suitable strain for the UCMS regimen and we also aimed to investigate the effects of different kind of antidepressants in this model.

4.1.1. Coat State

Fig.14A illustrates the total score of the coat state in BALB/c mice. Kruskal Wallis H showed a significant effect for coat state ($H=23.487$, $P=0.000$). We observed a significant difference between non-stressed and stressed vehicle groups in BALB/c mice ($P=0.001$). In contrast, there are no significant differences between groups in Swiss mice ($H= 8.301$, $P=0.140$) (Fig.14B). Fluoxetine and maprotiline significantly antagonized the degradation induced by the UCMS regimen in BALB/c mice ($P=0.033$, $P=0.033$, respectively). However, they did not elicit significant effects in Swiss mice. In the second experiment that we realized to evaluate the effects of desipramine, the test of Kruskal-Wallis revealed a significant difference between the groups in BALB/c mice ($H=18.19$, $P=0.000$, Fig.15A) and also in Swiss mice ($H=8.262$, $P=0.0418$, Fig.15B). Desipramine induced a significant improvement of the coat state of the mice when compared to the vehicle group in BALB/c ($P=0.005$) mice but not in Swiss mice ($P=0.296$). The effects of imipramine on the coat state in BALB/c and Swiss mice are shown in the Fig.16A and B, respectively. We observed significant differences between groups in BALB/c mice ($H=28.860$, $P=0.000$) but not in Swiss mice ($H=5.519$, $P=0.138$).

Imipramine treatment significantly ameliorated the coat state of the mice when compared to the control group ($P=0.013$) only in BALB/c mice. We did not observe any drug effects in the non-stressed groups when compared to the vehicle group in both strains in this experiments.

4.1.2. Splash Test

The effects of fluoxetine and maprotiline on the frequency of the grooming behaviour in the splash test in BALB/c mice and Swiss mice are shown in Fig.14C and D, respectively. The test of Kruskal-Wallis H revealed a significant difference between the groups for BALB/c (H=11.395, P=0.044) but not for Swiss mice (H=8.218, P=0.145). Non-stressed BALB/c mice groomed significantly more than stressed mice (P=0.011). Fluoxetine significantly diminished the effects of UCMS regimen on the grooming behaviour in BALB/c mice (P=0.042) but not Swiss mice whereas maprotiline had no effect in both strains. As shown in Fig.15C and D, desipramine augmented robustly the grooming behaviour when compared to the vehicle group in stressed BALB/c mice (P=0.001) but not in Swiss mice (H=2.112, P=0.550).

The effect of imipramine on the grooming behaviour is displayed in Fig.16C and D. By the Kruskal-Wallis H test, we observed a significant difference between all the groups in BALB/c mice (H=12.986, P=0.005) but not Swiss mice (H=2.920, P=0.404). Imipramine increased significantly the grooming behaviour when compared to the vehicle group in stressed BALB/c mice (P= 0.003). Any of the antidepressants that we used during the study revealed significant effects in the non-stressed groups when compared to the non-stressed vehicle in both strain.

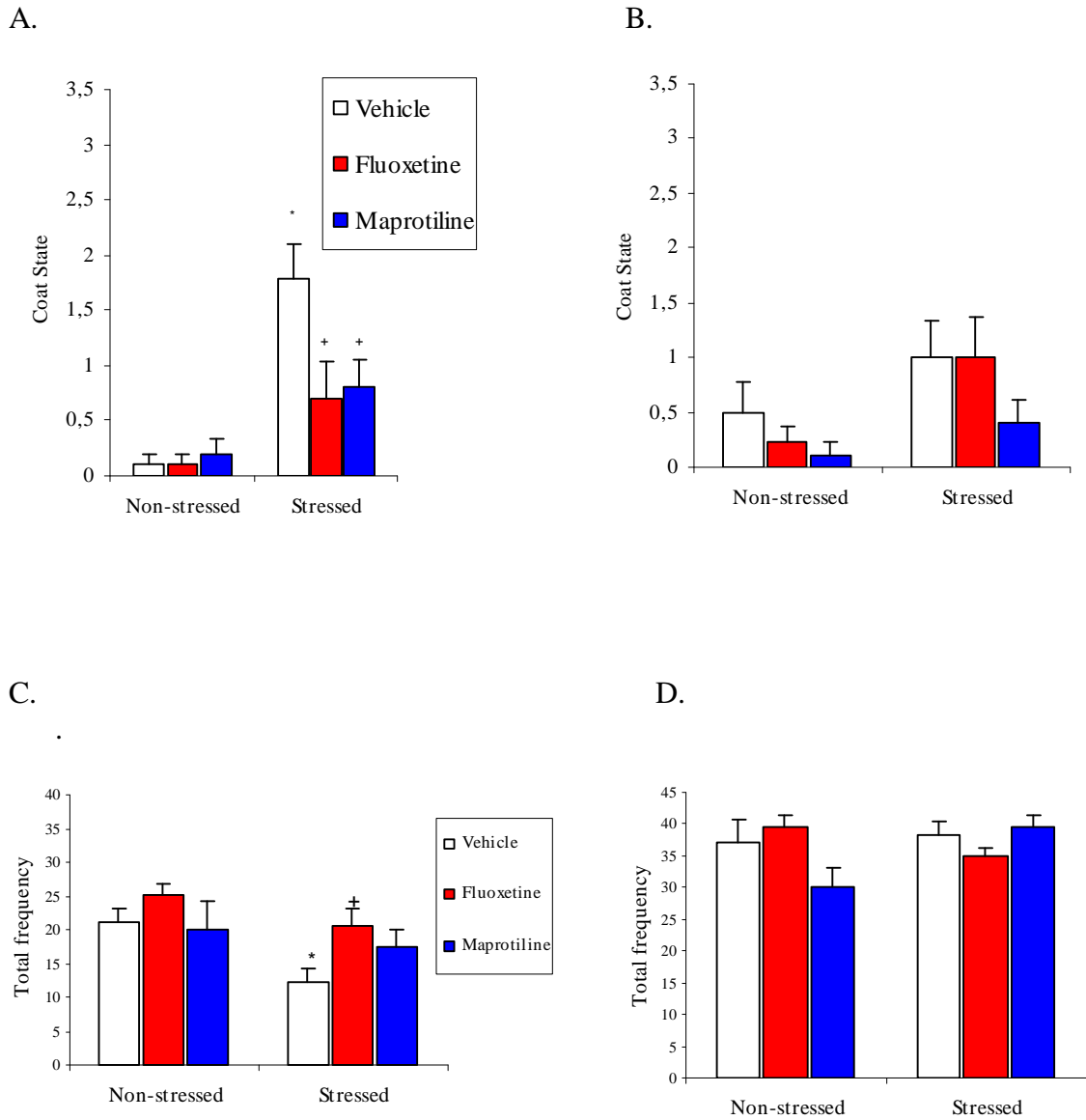


Fig.14. Effects of UCMS regimen and antidepressants; fluoxetine (10 mg/kg, i.p) and maprotiline (5 mg/kg, i.p) on the coat state in BALB/c mice (A) or in Swiss mice (B) and on the grooming behaviour in the splash test in BALB/c mice (C) or in Swiss mice (D). * $P < 0.05$ significantly different when compared to the non-stressed vehicle (0.9% NaCl), + $P < 0.05$ significantly different when compared to the stressed vehicle. All of the treatments begun after two weeks of stress regimen and were administered during 4 weeks. Data are shown as the means \pm S.E.M.

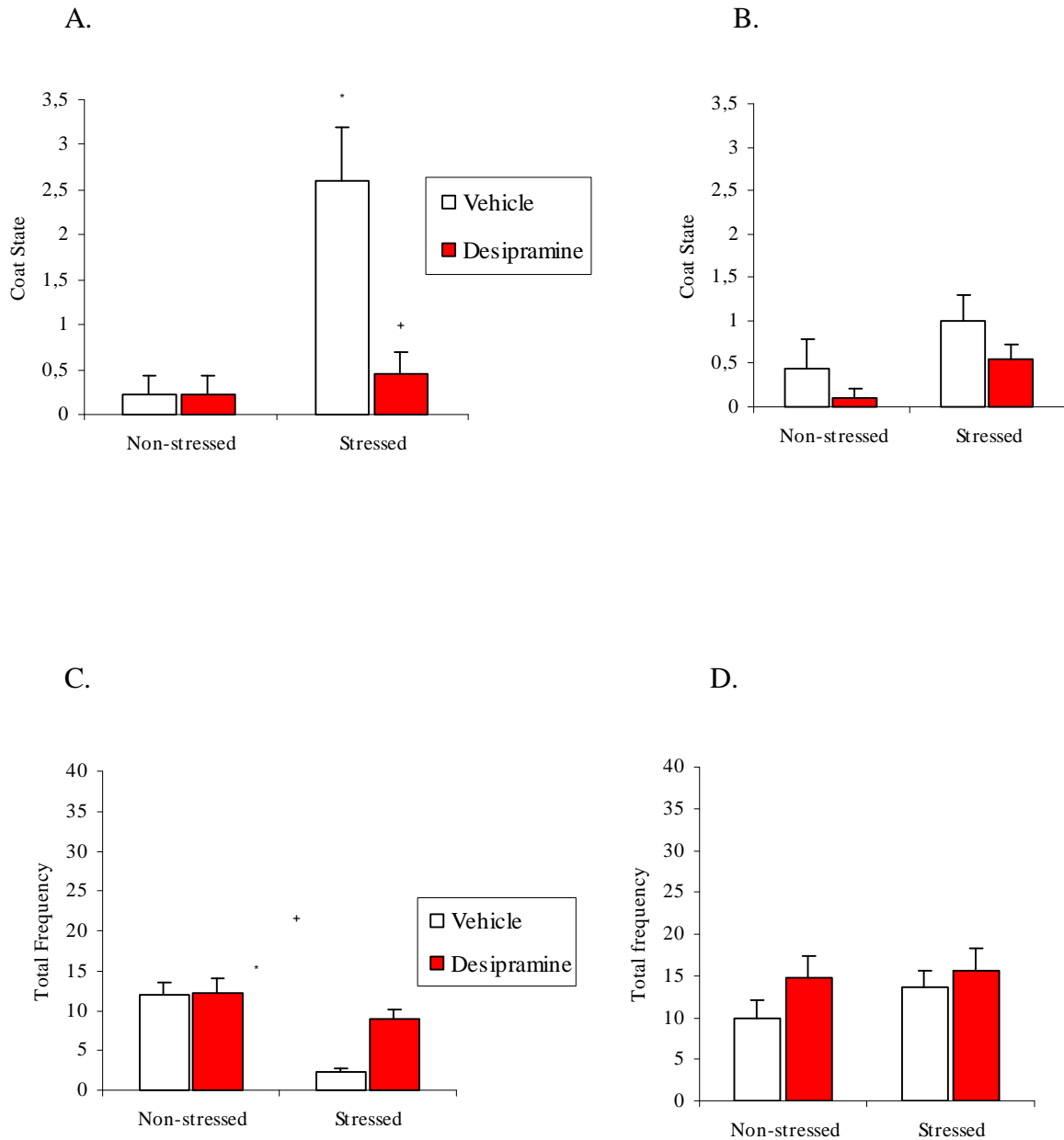


Fig.15. Effects of UCMS regimen and desipramine (10 mg/kg, i.p) on the coat state in BALB/c mice (A) or in Swiss mice (B) and on the grooming behaviour in the splash test in BALB/c mice (C) or in Swiss mice (D). * $P < 0.05$ significantly different when compared to the non-stressed vehicle (0.9% NaCl), + $P < 0.05$ significantly different when compared to the stressed vehicle. All of the treatments begun after two weeks of stress regimen and were administered during 4 weeks. Data are shown as the means \pm S.E.M.

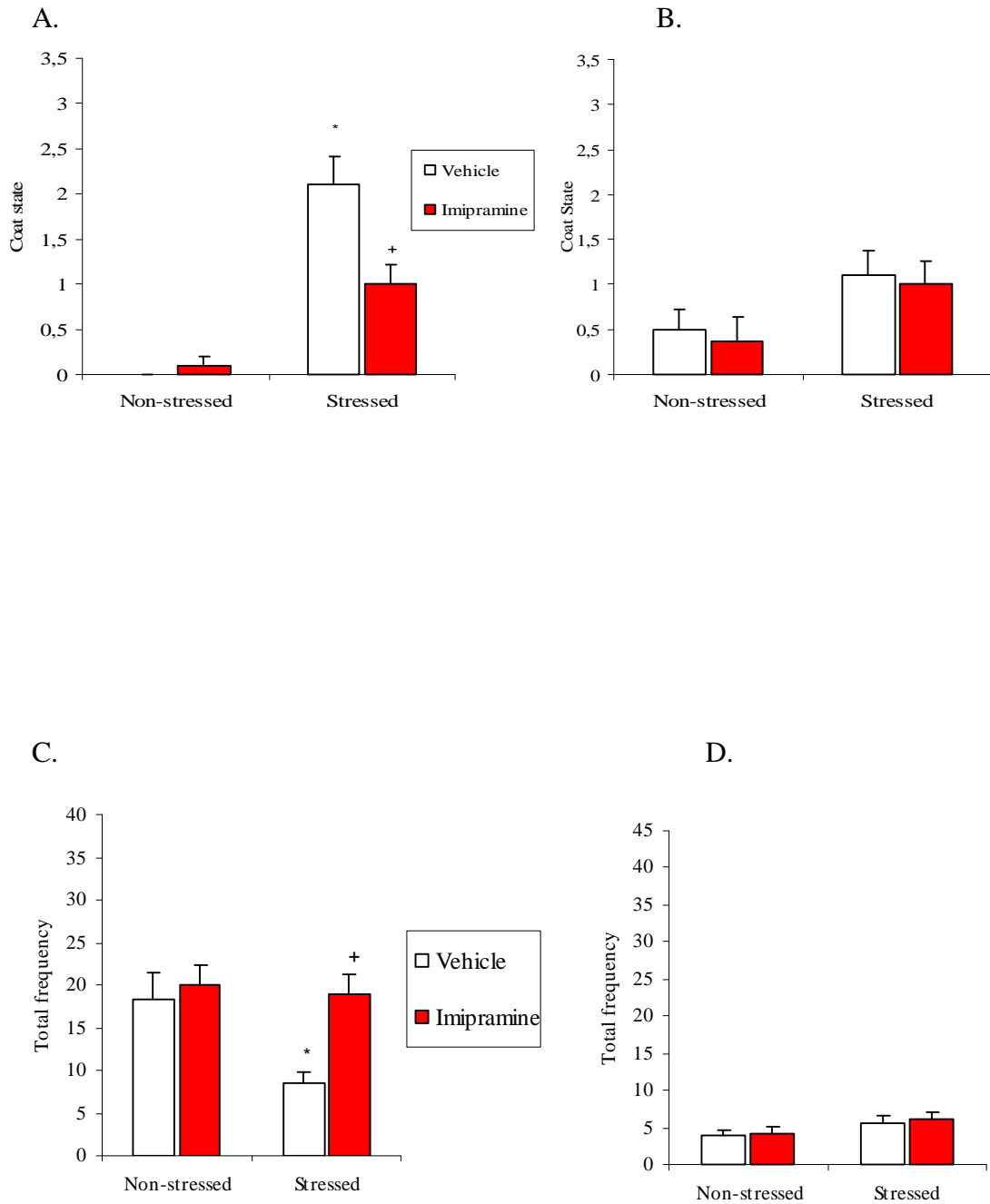


Fig.16. Effects of UCMS regimen and imipramine (20 mg/kg, i.p) on the coat state in BALB/c mice (A) or in Swiss mice (B) and on the grooming behaviour in the splash test in BALB/c mice (C) or in Swiss mice (D). * $P < 0.05$ significantly different when compared to the non-stressed vehicle (0.9% NaCl), + $P < 0.05$ significantly different when compared to the stressed vehicle. All of the treatments begun after two weeks of stress regimen and were administered during 4 weeks. Data are shown as the means \pm S.E.M.

4.1.3. Body Weight

We did not observe a significant difference between groups in BALB/c and Swiss mice in the experiments that we determined the action of fluoxetine and maprotiline (H=6.678, P=0.246, H=5.108, P=0.403, respectively, Table 8), desipramine (H=4.491, P=0.213, H=6.60, P=0.086, respectively, Table 9) and imipramine (H=4.66, P=1.98, H=2.23, P=0.525, respectively, Table 10)

4.1.4. Locomotor Activity

By the Kruskal-Wallis Test, no difference was observed between groups neither in BALB/c nor Swiss mice for the experiments that we searched the effects of fluoxetine and maprotiline (H=1.941, P=0.257, H=5.643, P=0.342, respectively, Table 8), desipramine (H=6.425, P=0.093, H=2.51, P=0.472, respectively, Table 9) and imipramine (H=1.003, P=0.801, H=6.10, P=0.107, respectively, Table 10).

Table 8. The effects of UCMS and four week drug treatment (Fluoxetine: 10 mg/kg, i.p, Maprotiline: 5 mg/kg, i.p) on the body weight and locomotor activity in BALB/c or Swiss mice. Results are shown as the means \pm S.E.M.

Strain	Environment	Treatment	Body Weight	Locomotor Activity
BALB/c	Non-stressed	Vehicle	30.39 \pm 0.58	2253.1 \pm 232.29
BALB/c	Non-stressed	Fluoxetine	30.57 \pm 0.65	1980.2 \pm 246.48
BALB/c	Non-stressed	Maprotiline	30.92 \pm 0.53	1815.4 \pm 173.24
BALB/c	Stressed	Vehicle	29.36 \pm 0.48	2003.77 \pm 182.62
BALB/c	Stressed	Fluoxetine	29.1 \pm 0.70	1840.8 \pm 206.70
BALB/c	Stressed	Maprotiline	30.02 \pm 0.37	2170.33 \pm 333.63
Swiss	Non-stressed	Vehicle	42.17 \pm 1.38	1028.5 \pm 189.5

Table 8. (Continue) The effects of UCMS and four week drug treatment (Fluoxetine: 10 mg/kg, i.p, Maprotiline: 5 mg/kg, i.p) on the body weight and locomotor activity in BALB/c or Swiss mice. Results are shown as the means \pm S.E.M.

Strain	Environment	Treatment	Body Weight	Locomotor Activity
Swiss	Non-stressed	Fluoxetine	43.02 \pm 1.07	1016.25 \pm 259.9
Swiss	Non-stressed	Maprotiline	42.42 \pm 0.74	1333.11 \pm 282.87
Swiss	Stressed	Vehicle	41.27 \pm 1.10	1684.3 \pm 232.10
Swiss	Stressed	Fluoxetine	42.02 \pm 1.21	1085.6 \pm 159.11
Swiss	Stressed	Maprotiline	40.51 \pm 0.78	1060.4 \pm 187.32

Table 9. The effects of UCMS and four week drug treatment (Desipramine: 10 mg/kg, i.p) on the body weight and locomotor activity in BALB/c or Swiss mice. Results are shown as the means \pm S.E.M.

Strain	Environment	Treatment	Body Weight	Locomotor Activity
BALB/c	Non-stressed	Vehicle	29.45 \pm 0.35	3359.6 \pm 265.53
BALB/c	Non-stressed	Desipramine	27.53 \pm 0.66	3332.6 \pm 269.2
BALB/c	Stressed	Vehicle	29.76 \pm 0.34	2255.4 \pm 300.56
BALB/c	Stressed	Desipramine	28.4 \pm 0.51	3195.88 \pm 379.77
Swiss	Non-stressed	Vehicle	44.03 \pm 0.86	970.5 \pm 140
Swiss	Non-stressed	Desipramine	41.46 \pm 0.79	1436.77 \pm 217.46
Swiss	Stressed	Vehicle	42.38 \pm 0.82	1113.3 \pm 236.31
Swiss	Stressed	Desipramine	40.67 \pm 0.46	1328.4 \pm 191.33

Table10. The effects of UCMS and four week drug treatment (Imipramine: 20 mg/kg, i.p) on the body weight and locomotor activity in BALB/c or Swiss mice. Results are shown as the means \pm S.E.M.

Strain	Environment	Treatment	Body Weight	Locomotor Activity
BALB/c	Non-stressed	Vehicle	32.02 \pm 0.87	2447 \pm 214.97
BALB/c	Non-stressed	Imipramine	31.39 \pm 0.44	2860 \pm 309.96
BALB/c	Stressed	Vehicle	29.73 \pm 0.69	2737 \pm 310.43
BALB/c	Stressed	Imipramine	30.7 \pm 0.56	2850 \pm 293.14
Swiss	Non-stressed	Vehicle	40.4 \pm 0.83	1075.3 \pm 257.37
Swiss	Non-stressed	Imipramine	41.46 \pm 0.79	1057.875 \pm 141.38
Swiss	Stressed	Vehicle	42.38 \pm 0.82	1185.22 \pm 171.29
Swiss	Stressed	Imipramine	40.67 \pm 0.46	1629 \pm 108.75

4.2. The Effects of Tramadol and Desipramine in an UCMS Model in BALB/c Mice

We designed this part to examine the possible antidepressant-like effects of tramadol in the UCMS.

4.2.1. Coat State

Figure 17A and B illustrates the total score of the coat state during six weeks and six weeks after the beginning of the UCMS, respectively. The test of Kruskal-Wallis H revealed a significant difference between the groups from the beginning of the fourth week (1 week after the drug treatment) until the sixth weeks ($H=16.211$, $P=0.006$, $H=13.284$ $P=0.0021$, $H=20.989$, $P=0.001$, respectively)(Fig.17A). We also observed a significant difference between non-stressed vehicle and stressed vehicle groups from the beginning of the fourth week until the end of the UCMS regimen ($P=0.003$, $P=0.022$, $P=0.001$). The drugs effects were observed only from the beginning of the sixth week. Tramadol (20 mg/kg, $P=0.005$) and desipramine (10 mg/kg, $P=0.015$) significantly reversed the degradation on the coat state induced by UCMS in stressed mice when compared to vehicle group after the 3 weeks treatment (Fig.17B).

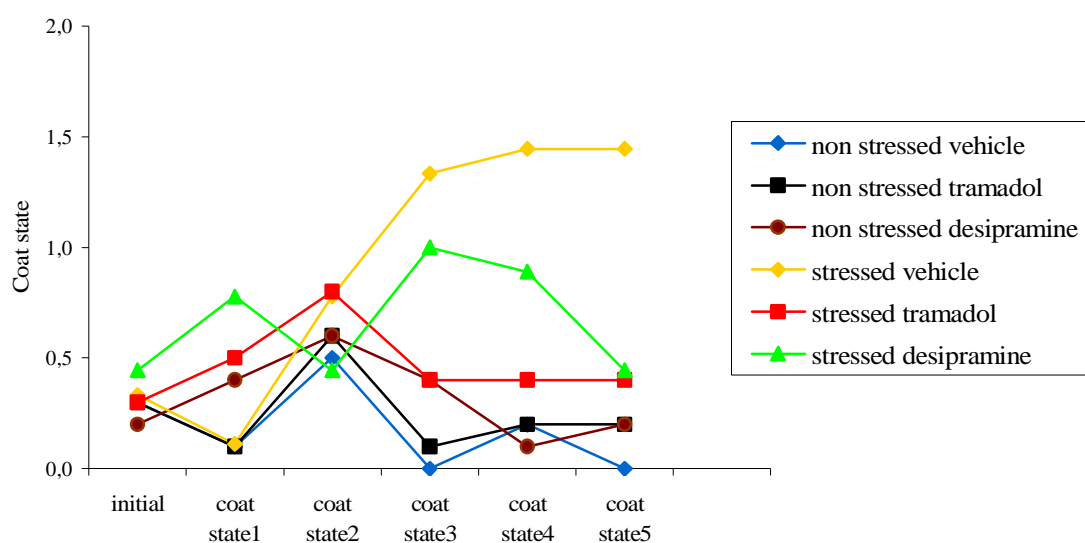


Fig.17A. Effects of desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) on the coat state in non-stressed and stressed groups during the 4 weeks of treatment. Initial state shows the state of the coat before the UCMS regimen. Coat state 1 shows the state of the coat of mice after the one week of stress procedure. The standard errors are not shown in the figure to make it more clear.

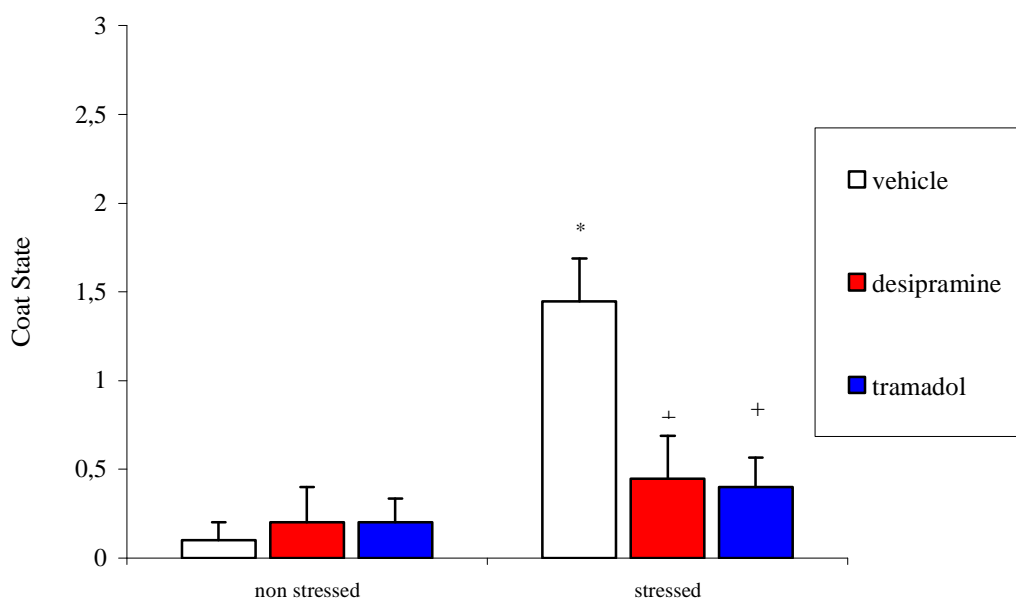


Fig.17B. Effects of desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) on the coat state in non stressed and stressed groups after the end of the UCMS regimen. All of the treatments begun after two weeks of stress regimen and were administered during 4 weeks.* $P < 0.05$, significantly different when compared to the non stressed vehicle (0.9% NaCl, i.p), + $P < 0.05$, significantly different when compared to the stressed vehicle. Results are shown as the means \pm S.E.M.

4.2.2. Splash Test

The effects of tramadol and desipramine on the grooming frequency are shown in Fig.18. By the Kruskal-Wallis H test, we observed a significant difference between all the groups ($H=13.504$, $P=0.019$). Non-stressed mice groomed significantly more than stressed mice ($P=0.011$). Both tramadol ($P=0.007$) and desipramine ($P=0.002$) significantly augmented the frequency of grooming behaviour in stressed mice in the splash test, but they did not elicit any effect in non-stressed mice ($P=0.389$, $P=0.411$, respectively).

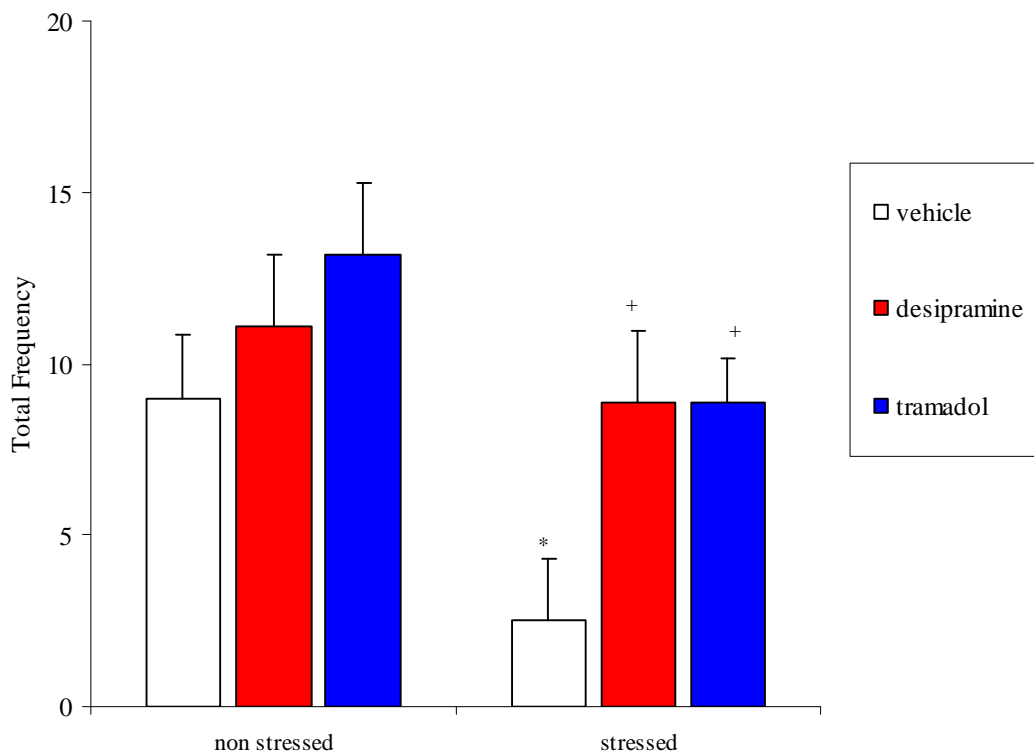


Fig.18. Effects of a 4 week treatment with desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) on the total frequency of the grooming behaviour during the splash test after the end of the UCMS regimen. All of the treatments begun after two weeks of stress regimen and were administered during 4 weeks. * $P<0.05$, significantly different when compared to the non-stressed vehicle, + $P<0.05$, significantly different when compared to the stress vehicle. Results are shown as the means \pm S.E.M.

4.2.3. Body Weight

There is a significant difference between groups ($H=15.37$, $P=0.009$). We did not observe any significant difference between the body weight of non-stressed and stressed mice ($P= 0.391$). Furthermore tramadol and desipramine treatment diminished the body weight when compared with vehicle in non-stressed (P values for tramadol; $P=0.006$, for desipramine $P=0.037$) and stressed mice (P values for tramadol; $P=0.041$, for desipramine $P=0.038$) (Table 11).

4.2.4. Locomotor Activity

No significant impairment of locomotor activity due to UCMS regimen or treatment was observed ($H=9.481$, $P=0.091$). These results were presented in the Table 11.

Table 11. The effects of UCMS and drugs treatment (Desipramine: 10 mg/kg, i.p, Tramadol: 20 mg/kg, i.p) on the body weight and locomotor activity. All of the treatments begun after two weeks of stress regimen and were administered during 4 weeks. * $P<0.05$, significantly different when compared to the non-stressed vehicle, + $P<0.05$, significantly different when compared to the stress vehicle. Results are shown as the means \pm S.E.M.

Environment	Treatment	Body Weight	Locomotor Activity
Non-stressed	Vehicle	29.25 ± 0.38	3275.10 ± 252.11
Non-stressed	Desipramine	$27.66 \pm 0.60^*$	3350.40 ± 241.43
Non-stressed	Tramadol	$27.63 \pm 0.34^*$	2634.30 ± 328.12
Stressed	Vehicle	29.77 ± 0.35	2255.44 ± 283.07
Stressed	Desipramine	$28.41 \pm 0.51^+$	3195.88 ± 379.77
Stressed	Tramadol	$28.51 \pm 0.41^+$	2942.40 ± 228.29

4.2.5. Morris Water Maze

No significant changes were observed between groups by Kruskal Wallis H test during the familiarization part ($H=2.860$, $P=0.749$) or the 4 sessions of the learning part ($H=4.515$, $P=0.478$; $H=8.815$, $P=0.115$; $H=7.548$, $P=0.183$, $H=3.665$, $P=0.599$) (data not shown). In contrast, during the learning part, the time to find the platform decreased from the first to the last session for all the groups including non-stressed and stressed mice (For vehicle $H=14.70$, $P=0.002$, tramadol $H=12.45$, $P=0.006$, desipramine $H=10.45$, $P=0.015$ in non-stressed mice, for vehicle $H=16.962$, $P=0.001$, tramadol $H=15.71$, $P=0.00$, desipramine $H=17.16$, $P=0.001$ in stressed mice) (Fig.19).

In the probe test session, both UCMS and drug treatment did not alter the time spent in the W quadrant ($H=3.518$, $P=0.621$) (data not shown).

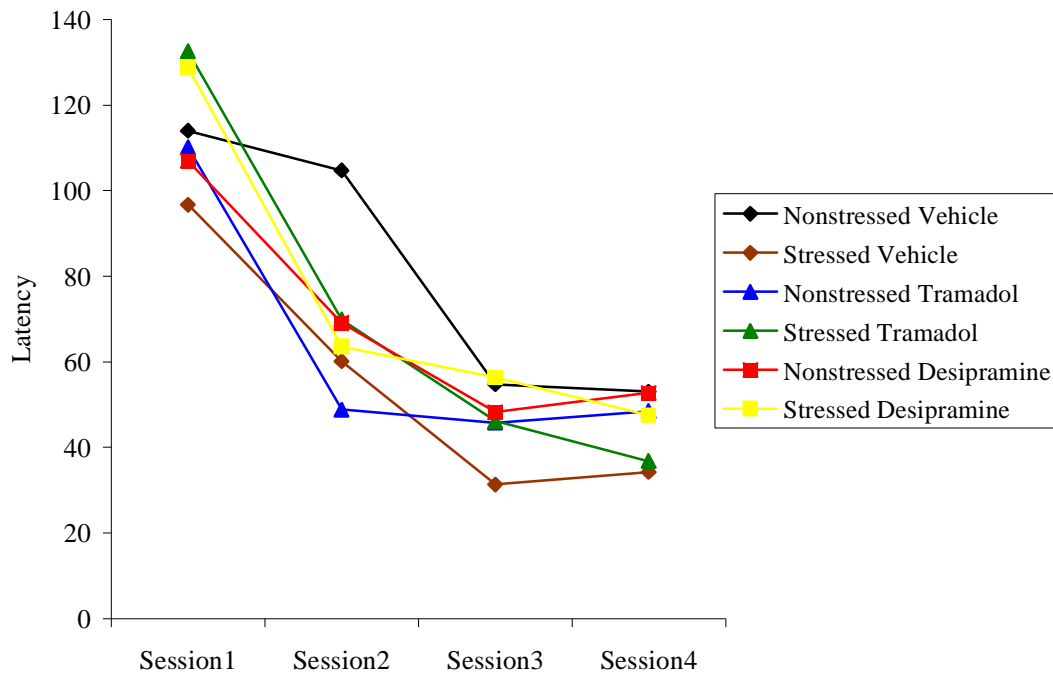


Fig.19. The effects of UCMS and drug treatment on the latency of the session of the learning part in the beginning of the sixth weeks in water maze. Values are means. The standard errors are not indicated in order to clarify the figure.

4.3. The Contribution of the Serotonergic System to the Antidepressant-like Effects of Tramadol and Desipramine in UCMS Model in BALB/c Mice

We aimed to clarify the mechanisms underlying the antidepressant-like action of tramadol; the participation of serotonergic system especially the effects of 5-HT_{1A} receptors by the 5-HT_{1A} receptor antagonist pindolol was searched in this part

4.3.1. The Effect of the Administration of 5, 7-Dihydroxytryptamine (5, 7-DHT) into Dorsal Raphe Nucleus on the Antidepressant-like Effects of Desipramine and Tramadol in the UCMS Model

4.3.1.1. Behavioural Tests

4.3.1.1.1. Coat State

The effects of a 5,7-DHT induced lesion in the DRN on the antidepressant-like action of desipramine and tramadol over the coat state are shown in Fig.20. Kruskal-Wallis H showed a significant effect for coat state ($H=48.57$, $P=0.000$). No significant differences were observed between lesioned and sham in non-stressed mice ($P=0.526$).

Also, we did not observe any differences between the lesioned and sham desipramine ($P=0.715$) or lesioned and sham tramadol ($P=0.542$) group in non-stressed mice. On the other hand, a significant degradation of the coat state of the mice was observed between non-stressed and stressed lesioned ($P=0.007$) or sham ($P=0.000$) mice.

However, even if the degradation of the coat state of the mice was significantly improved by the chronic treatment of desipramine ($P=0.001$) or tramadol ($P=0.004$) in stressed sham mice, these drugs did not achieve to counteract the effects of stress in lesioned mice ($P=0.458$ for desipramine, $P=0.086$ for tramadol). Moreover, the coat state of lesioned tramadol did not differ from sham tramadol group in stressed mice (0.335). In addition, there were no differences between lesioned desipramine and sham desipramine group in stressed mice ($P=0.2$)

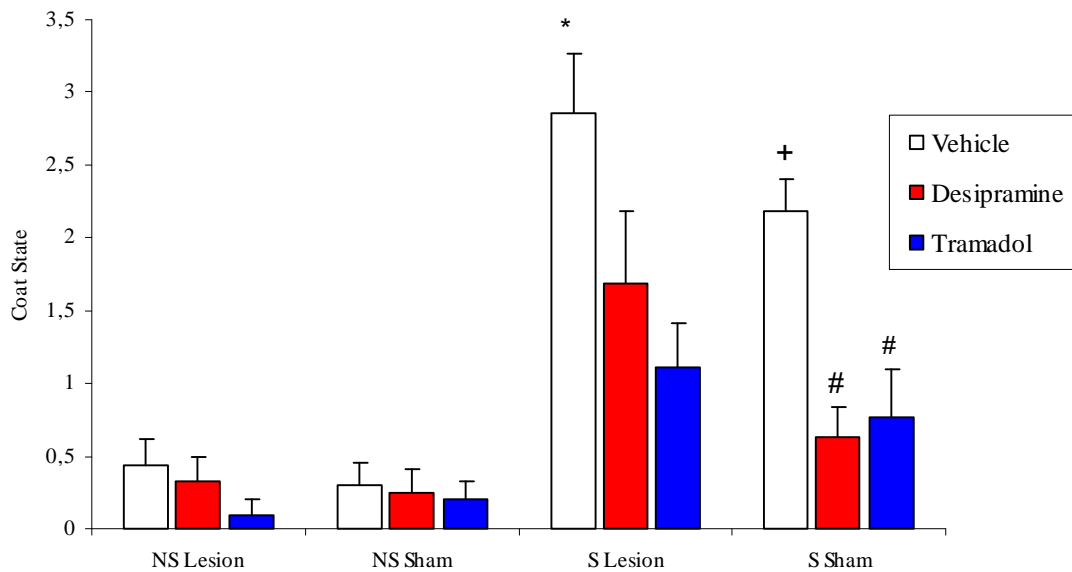


Fig.20. Effects of 5, 7-DHT lesions on the UCMS regimen and on the antidepressant-like action of tramadol (20 mg/kg, i.p) and desipramine (10 mg/kg, i.p) over the coat state in non-stressed (NS) and stressed (S) mice. Sham group received 0.2% ascorbic acid and 5, 7 DHT (1µg/0.2µl) was administered in DRN for the lesion groups. * P<0.05, significantly different when compared to the NS lesioned vehicle, + P<0.05, significantly different when compared to the NS sham vehicle, # P<0.05, significantly different when compared to the S sham vehicle group. All of the treatments begun after two weeks of stress regimen and were administered during 4 weeks. Results are shown as the means ± S.E.M.

4.3.1.1.2. Splash Test

The effects of 5, 7-DHT lesion on the grooming frequency in the non-stressed and stressed mice are shown in the Fig.21. By the Kruskal-Wallis H test, we observed a significant difference between groups (H=19.42, P=0.05). Non-stressed lesioned mice groomed significantly more than the stressed lesioned mice (P=0.009). In addition, the stress exposure diminished the grooming behaviour in stressed sham mice when compared to non-stressed sham mice but this decrease was not significant statistically (P=0.137). Both tramadol (P=0.029) and desipramine (P=0.045) significantly augmented the frequency of the grooming behaviour in stressed sham mice when compared to vehicle group, but they did not reverse the effects of stress in the lesioned mice (P=0.141 for tramadol, P=0.222 for desipramine).

No significant differences were observed between lesioned vehicle and sham vehicle in stressed mice (P=0.397).

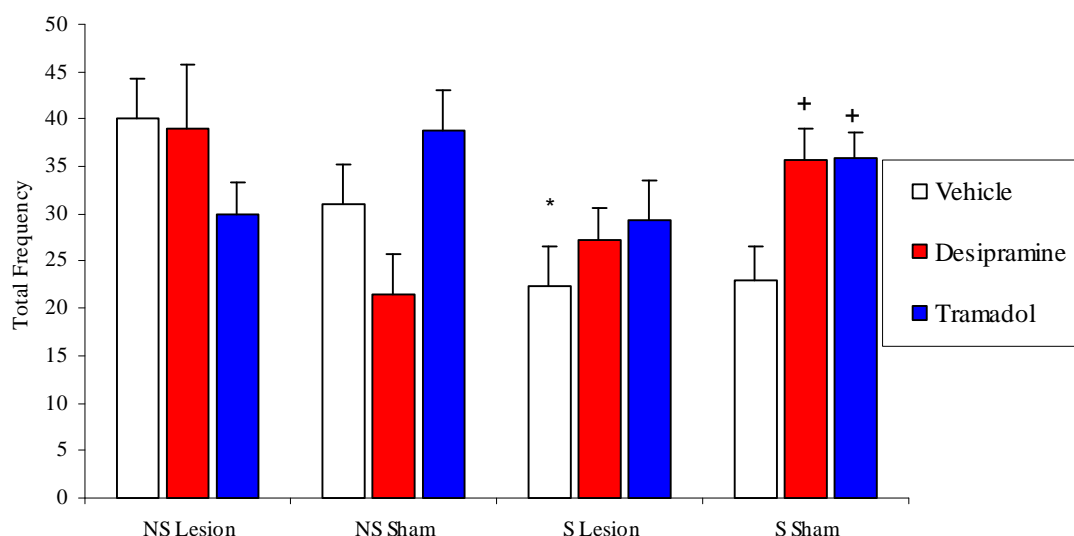


Fig.21. Effects of 5, 7-DHT lesion on the UCMS regimen and on the antidepressant-like action of tramadol (20 mg/kg, i.p) and desipramine (10 mg/kg, i.p) on the total frequency of the grooming behaviour during the splash test in non-stressed (NS) and stressed (S) mice. Sham group received 0.2% ascorbic acid and 5, 7-DHT (1µg/0.2µl) was administered in DRN for the lesion groups. * P<0.05, significantly different when compared to the NS lesioned vehicle, + P<0.05, significantly different when compared to the S sham vehicle group. All of the treatments begun after two weeks of stress regimen and were administered during 4 weeks. Results are shown as the means ± S.E.M.

4.3.1.1.3. Resident-Intruder Test

Kruskal Wallis H revealed significant effects between groups for the attack latency (H=38.75, P=0.000). The attack latency was significantly reduced in UCMS-treated animals in both lesioned and sham mice when compared to non-stressed vehicle mice. Tramadol or desipramine exposure significantly augmented the attack latency both in sham (P=0.003, P=0.000, respectively) and lesioned (P=0.005, P=0.017, respectively) stressed mice when compared to sham or lesioned vehicle group (Fig.22).

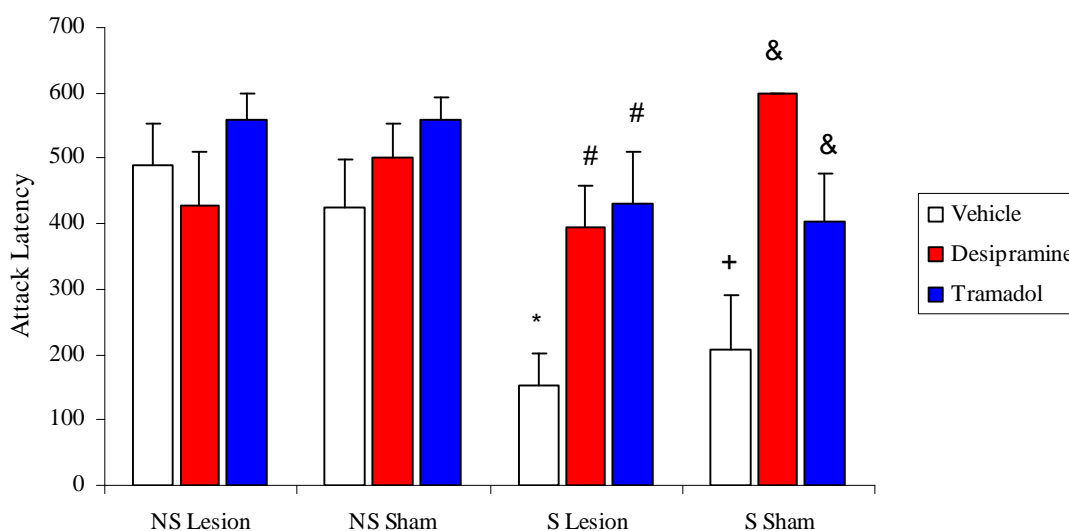


Fig.22. Effects of 5, 7-DHT lesion on the UCMS regimen and on the antidepressant-like action of tramadol (20 mg/kg, i.p) and desipramine (10 mg/kg, i.p) on the latency of attack in the resident-intruder test in non-stressed (NS) and stressed (S) mice. Sham group received 0.2% ascorbic acid and 5, 7-DHT (1µg/0.2µl) was administered in DRN for the lesion groups. * P<0.05, significantly different when compared to the NS lesioned vehicle, + P<0.05, significantly different when compared to the NS sham vehicle, # P<0.05, significantly different when compared to the S lesioned vehicle, & P<0.05, significantly different when compared to the S sham vehicle group. All of the treatments begun after two weeks of stress regimen and were administered during 4 weeks. Results are shown as the means ± S.E.M.

4.3.1.1.4. Body Weight and Locomotor Activity

We did not observe any effect of 5, 7-DHT lesion in non-stressed or stressed mice on the body weight (H=6.875, P=0.809) and on the locomotor activity (H=10.97, P=0.446) (Table 12).

Table 12. The effects of 5, 7-DHT, UCMS and drugs (Desipramine: 10 mg/kg, i.p, Tramadol: 20 mg/kg, i.p) on the body weight and locomotor activity. All of the treatments begun after two weeks of stress regimen and were administered during 4 weeks. Results are shown as the means \pm S.E.M.

Environment	Operation	Treatment	Body Weight	Locomotor Activity
Non-stressed	Lesion	Vehicle	32.05 \pm 0.67	2011.9 \pm 215.25
Non-stressed	Lesion	Desipramine	30.94 \pm 0.84	1741.88 \pm 292.17
Non-stressed	Lesion	Tramadol	30.3 \pm 0.84	1953.8 \pm 320.11
Non-stressed	Sham	Vehicle	30.35 \pm 0.55	2053.4 \pm 270.71
Non-stressed	Sham	Desipramine	30.9 \pm 1.17	2368,87 \pm 232.17
Non-stressed	Sham	Tramadol	30.7 \pm 0.98	1586.7 \pm 94.85
Stressed	Lesion	Vehicle	31.09 \pm 0.46	2206.33 \pm 198.14
Stressed	Lesion	Desipramine	30.9 \pm 1.17	1617.23 \pm 206.51
Stressed	Lesion	Tramadol	30.8 \pm 0.77	2007.33 \pm 289.29
Stressed	Sham	Vehicle	30.22 \pm 0.7	2199.4 \pm 309.77
Stressed	Sham	Desipramine	30.45 \pm 0.6	2287.81 \pm 258.88
Stressed	Sham	Tramadol	29.94 \pm 0.59	2167.11 \pm 259.78

4.3.1.2. Biochemistry

4.3.1.2.1. 5-HT Content

The mean and standard errors for the 5-HT levels in the cerebellum, striatum, frontal cortex, hippocampus and the region of raphe magnus in μ M/g tissue weight for lesioned and sham groups are presented in Table 13.

No significant effects between groups were observed for the level of 5-HT in the cerebellum (H=18.17, P=0.078).

In contrast, statistically, there was a significant main effect between groups in the striatum (H=28.79, P=0.002). In this area, the neurotoxin reduced the levels of 5-HT

in all the non-stressed groups. The level of 5-HT was significantly diminished by 5, 7-DHT in the lesioned group when compared to the sham vehicle group in non-stressed ($P=0.007$) and stressed ($P=0.006$) mice. The treatment with desipramine or tramadol did not prevent the action of 5, 7-DHT neither in non-stressed mice ($P=0.211$, $P=0.354$, respectively) nor in stressed mice ($P=0.122$, $P=0.189$, respectively). In the sham mice, the UCMS diminished the 5-HT level in control mice when compared to non-stressed mice ($P=0.04$). Neither desipramine nor tramadol changed the 5-HT level in the sham mice when compared to the vehicle group ($P=0.940$, $P=0.558$, respectively). In non-stressed mice, the level of 5-HT was also reduced by the neurotoxin in the tramadol or desipramine treated groups ($P=0.012$ and $P=0.008$, respectively).

The test of Kruskal-Wallis H revealed significant differences between groups ($H=29.27$, $P=0.002$) in the frontal cortex. The level of 5-HT was reduced by the 5, 7-DHT lesion between lesioned and sham non-stressed vehicle group ($P=0.003$). On the contrary, the lesion with 5, 7-DHT did not induce any significant reduction in the level of 5-HT in any other groups. 5-HT level was significantly reduced by the UCMS regimen in sham mice ($P=0.009$). Only tramadol augmented the 5-HT level in the sham stressed mice when compared to vehicle group ($P=0.004$).

The Kruskal-Wallis H test showed a significant difference between groups for the 5-HT level in hippocampus ($H=25.7$, $P=0.007$). The concentration of 5-HT was significantly reduced by the 5, 7-DHT lesion in non-stressed vehicle mice ($P=0.026$). In addition, the level of 5-HT level also significantly diminished in the non stressed mice treated with desipramine ($P=0.02$). However, tramadol augmented the level of 5-HT in sham stressed mice ($P=0.033$), desipramine augmented 5-HT concentration in lesioned stressed mice ($P=0.038$).

There was a significant difference between groups in the region of the raphe magnus ($H=24.63$, $P=0.01$). The level of 5-HT was reduced by the lesion in the tramadol treated stressed mice ($P=0.015$). 5, 7-DHT lesion slightly diminished the level of 5-HT in the non-stressed vehicle group but the difference was not significant. In addition, the stress exposure diminished the 5-HT level in the sham stressed mice when compared to non-stressed mice ($P=0.024$). This diminution was counteracted by the chronic treatment with tramadol ($P=0.007$) when compared to vehicle stressed mice.

Table 13. Effect of 5, 7-DHT lesion on the 5-HT level in the cerebellum (CRB), striatum (STR), frontal cortex (FC), hippocampus (HC) and the region of the raphe nucleus (RN) in the non-stressed (NS) and stressed (S) mice which were treated either vehicle (VHC) or drugs (tramadol (TRM) 20 mg/kg, i.p or desipramine 10 mg/kg, i.p (DMI)). The drugs were administered during 4 weeks. All data are expressed in terms of $\mu\text{M/g}$ tissue weight. Data are presented as means \pm S.E.M. * $P < 0.05$, significantly different when compared to the NS sham vehicle, # $P < 0.05$, significantly different when compared to the NS sham desipramine, & $P < 0.05$, significantly different when compared to the NS sham tramadol, + $P < 0.05$, significantly different when compared to the S sham vehicle, ^ $P < 0.05$, significantly different when compared to the S sham tramadol, \$ $P < 0.05$, significantly different when compared to the S lesioned vehicle. (Envir: Environment, Oper: Operation, Treat: Treatment)

Envir	Oper	Treat	CRB	STR	FC	HC	Region of RN
NS	Lesion	VHC	0,86 \pm 0,26	2,03 \pm 0,48*	1,82 \pm 0,33*	1,83 \pm 0,31*	4,39 \pm 0,61
NS	Lesion	DMI	0,85 \pm 0,23	1,23 \pm 0,52 [#]	2,70 \pm 0,22	1,70 \pm 0,53 [#]	3,67 \pm 0,39
NS	Lesion	TRAM	1,10 \pm 0,18	2,77 \pm 0,70 ^{&}	3,31 \pm 0,55	2,20 \pm 0,63	3,98 \pm 0,50
NS	Sham	VHC	0,91 \pm 0,29	4,65 \pm 0,47	4,15 \pm 0,55	3,70 \pm 0,60	6,62 \pm 1,08
NS	Sham	DMI	1,03 \pm 0,34	5,20 \pm 1,63	5,11 \pm 1,19	4,17 \pm 0,44	6,60 \pm 1,40
NS	Sham	TRAM	1,58 \pm 0,29	5,09 \pm 1,37	3,88 \pm 0,55	4,04 \pm 0,81	5,58 \pm 0,68
S	Lesion	VHC	0,61 \pm 0,11	1,40 \pm 0,41 ⁺	2,96 \pm 0,68	1,17 \pm 0,31	3,45 \pm 0,45
S	Lesion	DMI	0,85 \pm 0,15	2,14 \pm 0,35	1,92 \pm 0,22	2,89 \pm 0,50 ^{\$}	3,56 \pm 0,39
S	Lesion	TRAM	1,10 \pm 0,26	2,77 \pm 0,70	3,31 \pm 0,38	2,20 \pm 0,48 [^]	3,98 \pm 0,50 [^]
S	Sham	VHC	0,99 \pm 0,25	2,87 \pm 0,55*	2,23 \pm 0,30*	2,51 \pm 0,57	3,33 \pm 0,48*
S	Sham	DMI	1,36 \pm 0,30	3,32 \pm 0,94	3,13 \pm 0,62	3,13 \pm 0,60	5,60 \pm 0,82
S	Sham	TRAM	1,28 \pm 0,23	3,92 \pm 1,20	4,02 \pm 0,42 ⁺	4,82 \pm 0,78 ⁺	10,60 \pm 2,5 ⁺

4.3.1.2.2. 5-HIAA Content

Table 14 illustrates the effects of 5, 7-DHT lesion on the level of 5-HIAA in the non-stressed and stressed mice.

Kruskal-Wallis H did not reveal significant effects between groups for the 5-HIAA level in the cerebellum ($H=7.806$, $P=0.731$) and in the frontal cortex ($H=16.11$, $P=0.137$).

On the other hand, significant differences among groups appeared for the level of 5-HIAA in the striatum ($H=24.64$, $P=0.01$). 5, 7-DHT caused a significant reduction in 5-HIAA concentrations in the non-stressed vehicle ($P=0.005$) when compared to its sham group, while no significant difference observed between lesioned and sham stressed vehicle mice. However, the stress procedure diminished the level of 5-HIAA in sham mice when compared to non-stressed sham mice ($P=0.004$), tramadol significantly reversed this effect ($P=0.005$).

In addition, Kruskal Wallis test also revealed significant differences between the groups for the 5-HIAA level in the hippocampus ($H=24.18$, $P=0.012$). Significant diminution was induced by the neurotoxin in the non-stressed vehicle ($P=0.011$), desipramine ($P=0.007$) and tramadol ($P=0.041$) group when compared to their sham groups. No differences were seen for the other groups.

Furthermore, significant differences were observed between groups in the region of the raphe magnus ($H=22.31$, $P=0.02$). 5, 7 DHT caused a significant reduction in 5-HIAA levels in the non-stressed vehicle groups ($P=0.023$). Although, the 5, 7-DHT caused a significant reduction in 5-HIAA levels between lesioned and shamed non-stressed vehicle groups ($P=0.023$), we did not observe any significant difference between lesioned and sham stressed vehicle groups ($P=0.595$).

In this region, the UCMS diminished the 5-HIAA level in the sham stressed vehicle group when compared to sham stressed vehicle mice when compared to non-stressed mice ($P=0.027$). In addition, tramadol augmented the 5-HIAA level both in lesioned and sham stressed mice when compared to vehicle groups ($P=0.03$, $P=0.01$, respectively).

Table 14. Effect of 5,7-DHT lesion on the 5-HIAA level in the cerebellum (CRB), striatum (STR), frontal cortex (FC), hippocampus (HC) and the region of the raphe nucleus (RN) in the non-stressed (NS) and stressed (S) mice which were treated either vehicle (VHC) or drugs (tramadol (TRM) 20 mg/kg, i.p or desipramine (DMI), 10 mg/kg, i.p). The drugs were administered during 4 weeks. All data are expressed in terms of $\mu\text{M/g}$ tissue weight. Data are presented as means \pm S.E.M. * $P < 0.05$, significantly different when compared to the NS sham vehicle, # $P < 0.05$, significantly different when compared to the NS sham desipramine, & $P < 0.05$, significantly different when compared to the NS sham tramadol, + $P < 0.05$, significantly different when compared to the S sham vehicle, ^ $P < 0.05$, significantly different when compared to the S sham tramadol. (Envir: Environment, Oper: Operation, Treat: Treatment)

Envir	Oper	Treat	CRB	STR	FC	HC	Region of RN
NS	Lesion	VHC	0,71 \pm 0,91	1,68 \pm 0,81*	0,74 \pm 0,18	1,11 \pm 0,15*	1,48 \pm 0,10*
NS	Lesion	DMI	0,91 \pm 0,12	5,08 \pm 1,63	0,72 \pm 0,09	0,86 \pm 0,21#	1,80 \pm 0,12
NS	Lesion	TRAM	0,59 \pm 0,09	3,01 \pm 1,13	1,40 \pm 0,57	1,38 \pm 0,23&	2,13 \pm 0,42
NS	Sham	VHC	0,64 \pm 0,18	4,42 \pm 0,92	1,84 \pm 0,65	4,80 \pm 2,05	3,31 \pm 0,52
NS	Sham	DMI	0,62 \pm 0,18	11,67 \pm 4,9	1,40 \pm 0,38	2,26 \pm 0,23	3,31 \pm 0,87
NS	Sham	TRAM	0,74 \pm 0,15	8,24 \pm 3,04	1,59 \pm 0,36	2,08 \pm 0,29	2,72 \pm 0,39
S	Lesion	VHC	0,78 \pm 0,24	2,61 \pm 0,37	0,66 \pm 0,09	1,33 \pm 0,42	1,38 \pm 0,25
S	Lesion	DMI	0,77 \pm 0,17	2,19 \pm 0,69	1,07 \pm 0,38	2 \pm 0,52	1,50 \pm 0,21
S	Lesion	TRAM	0,8 \pm 0,19	2,78 \pm 0,50	0,93 \pm 0,16	1,65 \pm 0,50	2,02 \pm 0,40^
S	Sham	VHC	0,98 \pm 0,26	1,77 \pm 0,41*	1,06 \pm 0,15	2,15 \pm 0,36	1,62 \pm 0,3*
S	Sham	DMI	0,93 \pm 0,16	5,29 \pm 1,71	1,43 \pm 0,53	3,96 \pm 1,20	2,7 \pm 0,53
S	Sham	TRAM	0,59 \pm 0,21	4,18 \pm 0,64+	2,21 \pm 0,80	3,96 \pm 1,71	4,23 \pm 1,02+

4.3.1.2.3. NA Content

The effects of the lesion of the DRN by 5, 7-DHT on the level of NA in non-stressed and stressed mice are shown in the Table 15. Kruskal Wallis H revealed a significant difference between the groups for the NA level in the cerebellum ($H=32.76$, $P=0.001$). 5, 7-DHT lesion did not induce any changes on the level of NA in any of the

group in this area by the Mann-Whitney U test. On the other hand, the level of NA was reduced by UCMS both in lesioned ($P=0.02$) and sham mice ($P=0.009$). The drug treatments did not elicit any effects neither in lesioned nor sham mice.

Moreover, the Kruskal Wallis H showed significant differences between groups ($H=35.66$, $P=0.000$) in the NA level in the striatum. The 5, 7-DHT lesion did not show any significant effect on the NA level in the striatum both in non-stressed and stressed mice. However, the UCMS regimen diminished the NA level both in lesioned ($P=0.004$) and sham ($P=0.003$) mice.

Tramadol and desipramine treatment reversed the effects of UCMS in lesioned ($P=0.05$ for tramadol, $P=0.018$ for desipramine) when compared to their controls. Desipramine treatment was also capable to improve the effects of stress in sham mice ($P=0.017$).

In contrast, there was no significant difference between groups for the NA level in the frontal cortex ($H=16.77$, $P=0.115$).

We showed significant differences between groups by the Kruskal-Wallis H in the hippocampus for the NA level ($H=21.89$, $P=0.025$). The 5, 7-DHT lesion did not elicit any significant effect on the NA level in the hippocampus for any groups in both non-stressed and stressed mice. On the other hand, the UCMS regimen reduced the NA level both in lesioned ($P=0.015$) and sham ($P=0.017$) mice. Tramadol and desipramine administration counteracted the effects of UCMS in lesioned ($P=0.041$ for tramadol, $P=0.035$ for desipramine) and sham mice ($P=0.004$ for desipramine, $P=0.03$ for tramadol) when compared to their control.

Moreover, by the Kruskal Wallis H test, we showed significant differences between groups concerning the NA level within the raphe nucleus ($H=22.23$, $P=0.023$). 5, 7-DHT lesion did not induce any significant effect on the NA level in the region of the raphe magnus for any group both in non-stressed and stressed mice. However, the UCMS induced a significant reduction on the level of NA both in lesioned ($P=0.023$) and sham ($P=0.041$) mice. Chronic treatment with desipramine and tramadol reversed the effects of stress exposure in lesioned ($P=0.05$ for tramadol, $P=0.008$ for desipramine) and sham mice ($P=0.005$ for tramadol, $P=0.036$ for desipramine).

Table 15. Effect of 5, 7-DHT lesion on the NA level in the cerebellum (CRB), striatum (STR), frontal cortex (FC), hippocampus (HC) and the region of the raphe nucleus (RN) in the non-stressed (NS) and stressed (S) mice which were treated either vehicle (VHC) or drugs (tramadol (TRM) 20 mg/kg, i.p or desipramine (DMI) 10 mg/kg, i.p). The drugs were administered during 4 weeks. All data are expressed in terms of $\mu\text{M/g}$ tissue weight. Data are presented as means \pm S.E.M. * $P<0.05$, significantly different when compared to the NS sham vehicle, ** $P<0.05$, significantly different when compared to the NS lesioned vehicle, + $P<0.05$, significantly different when compared to the S sham vehicle, \$ $P<0.05$, significantly different when compared to the S lesioned vehicle. (Envir: Environment, Oper: Operation, Treat: Treatment)

Envir	Oper	Treat	CRB	STR	FC	HC	Region of RM
NS	Lesion	VHC	3,22 \pm 1,62	9,76 \pm 3,49	2,65 \pm 0,82	2,68 \pm 0,58	3,19 \pm 0,63
NS	Lesion	DMI	2,61 \pm 0,61	9,10 \pm 2,55	1,82 \pm 0,35	2,97 \pm 1,15	4,35 \pm 0,78
NS	Lesion	TRAM	2,76 \pm 0,43	7,20 \pm 0,32	2,28 \pm 0,85	3,32 \pm 0,54	3,91 \pm 0,85
NS	Sham	VHC	3,67 \pm 0,88	7,61 \pm 2,97	4,31 \pm 1,52	5,01 \pm 1,37	3,98 \pm 0,89
NS	Sham	DMI	1,84 \pm 0,40	6,97 \pm 2,03	3,46 \pm 0,85	3,56 \pm 0,71	2,77 \pm 0,57
NS	Sham	TRAM	2,86 \pm 0,69	8,72 \pm 1,51	4,15 \pm 1,35	2,35 \pm 0,47	3,14 \pm 0,49
S	Lesion	VHC	0,56 \pm 0,29**	0,88 \pm 0,38**	1 \pm 0,26	0,79 \pm 0,40**	1,15 \pm 0,42**
S	Lesion	DMI	0,90 \pm 0,37	3,23 \pm 1,11\$	1,51 \pm 0,35	3,49 \pm 1,13\$	4,24 \pm 0,71\$
S	Lesion	TRAM	0,97 \pm 0,33	3,42 \pm 1,24\$	2,22 \pm 0,55	3,42 \pm 1,49\$	3,66 \pm 1,11\$
S	Sham	VHC	0,73 \pm 0,32*	0,75 \pm 0,34*	1,08 \pm 0,32	0,83 \pm 0,35*	1,49 \pm 0,57*
S	Sham	DMI	1,62 \pm 0,72	4,52 \pm 1,42+	2,01 \pm 0,75	4,20 \pm 0,73+	3,45 \pm 0,70+
S	Sham	TRAM	0,89 \pm 0,37	1,81 \pm 0,34	2,08 \pm 0,85	3,28 \pm 1,29+	5,03 \pm 0,81+

4.3.1.2.4. DA Content

The effects of 5, 7-DHT lesion on the DA content in the non-stressed and stressed control and drugs treated mice are shown in Table 16. The level of dopamine did not show significant differences between groups in the cerebellum ($H=8.03$,

P=0.71), the frontal cortex (H=13.56, P=0.258), the hippocampus (H=7.65, P=0.744) and the region of the raphe magnus (H=14.83, P=0.190).

In contrast, the Kruskal Wallis H revealed significant differences between groups in the striatum for the DA level (H=22.35, P=0.022). The 5, 7-DHT lesion did not induce any effect on the DA level in any groups in both non-stressed and stressed mice. On the other hand, the level of dopamine was diminished by the UCMS in lesioned (P=0.021) and sham (P=0.019) mice. In addition, chronic desipramine and tramadol treatment improved the effects of UCMS on the level of DA in lesioned mice (P=0.021 for tramadol, P=0.05 for desipramine). Tramadol also reversed the effects of UCMS significantly in the sham mice (P=0.05).

Table 16. Effect of 5, 7-DHT lesion on the DA level in the cerebellum (CRB), striatum (STR), frontal cortex (FC), hippocampus (HC) and the region of the raphe nucleus (RN) in the non-stressed (NS) and stressed (S) mice which were treated either vehicle (VHC) or drugs (tramadol (TRM) 20 mg/kg,i.p or desipramine (DMI) 10 mg/kg, i.p). The drugs were administered during 4 weeks. All data are expressed in terms of $\mu\text{M/g}$ tissue weight. Data are presented as means \pm S.E.M. * $P < 0.05$, significantly different when compared to the NS sham vehicle, ** $P < 0.05$, significantly different when compared to the NS sham vehicle, + $P < 0.05$, significantly different when compared to the S sham vehicle, \$ $P < 0.05$, significantly different when compared to the S lesioned vehicle. (Envir: Environment, Oper: Operation, Treat: Treatment)

Envir	Oper	Treat	CRB	STR	FC	HC	Region of RN
NS	Lesion	VHC	0,08 \pm 0,03	25,73 \pm 4,15	1,51 \pm 0,35	0,46 \pm 0,19	0,78 \pm 0,17
NS	Lesion	DMI	0,43 \pm 0,16	23,37 \pm 5,38	1,53 \pm 0,40	0,43 \pm 0,14	1,10 \pm 0,40
NS	Lesion	TRAM	0,34 \pm 0,12	25,15 \pm 4,40	2,48 \pm 0,59	0,35 \pm 0,20	1,67 \pm 0,51
NS	Sham	VHC	0,37 \pm 0,15	34,93 \pm 4,55	2,60 \pm 0,7	0,58 \pm 0,24	1,01 \pm 0,23
NS	Sham	DMI	0,44 \pm 0,28	34,47 \pm 6,55	2,39 \pm 0,77	0,39 \pm 0,11	1,67 \pm 0,31
NS	Sham	TRAM	0,35 \pm 0,11	38,61 \pm 6,82	3,62 \pm 1,28	0,50 \pm 0,16	1,01 \pm 0,18
S	Lesion	VHC	0,44 \pm 0,18	12,52 \pm 2,29 ^{**}	1,4 \pm 0,35	0,54 \pm 0,13	1,42 \pm 0,16
S	Lesion	DMI	0,43 \pm 0,20	23,37 \pm 3,81 ^{\$}	1,53 \pm 0,29	0,43 \pm 0,14	0,7 \pm 0,17
S	Lesion	TRAM	0,31 \pm 0,13	28,27 \pm 5,27 ^{\$}	2,09 \pm 0,49	0,57 \pm 0,28	0,66 \pm 0,63
S	Sham	VHC	0,47 \pm 0,11	19,26 \pm 3,42 [*]	1,2 \pm 0,3	0,29 \pm 0,09	1,10 \pm 0,27
S	Sham	DMI	0,22 \pm 0,07	18,94 \pm 4,19	1,69 \pm 0,59	0,76 \pm 0,22	2,52 \pm 1,30
S	Sham	TRAM	0,17 \pm 0,10	30,41 \pm 4,47 ⁺	2,32 \pm 0,71	0,89 \pm 0,29	1,48 \pm 0,27

4.3.1.2.5 Homovallinic Acid (HVA) Content

The effects of 5, 7-DHT lesion on the HVA level in non-stressed or stressed mice, treated or not are presented in Table 17.

The HVA level in the cerebellum and in the hippocampus are low and highly variable, thus they could not be determined. Further, the Kruskal Wallis H test did not

show any significant effect in the HVA level in the striatum (H=5.8, P=0.886), frontal cortex (H=4.55, P=0.95) and the region of the raphe magnus (H=8.11, P=0.703).

Table 17. Effect of 5, 7-DHT lesion on the HVA level in the cerebellum (CRB), striatum (STR), frontal cortex (FC), hippocampus (HC) and the region of the raphe nucleus (RN) in the non-stressed (NS) and stressed (S) mice which were treated either vehicle (VHC) or drugs (tramadol (TRM) 20 mg/kg, i.p or desipramine (DMI) 10 mg/kg, i.p). The drugs were administered during 4 weeks. All data are expressed in terms of $\mu\text{M/g}$ tissue weight. Data are presented as means \pm S.E.M. ND: Not determined. (Envir: Environment, Oper: Operation, Treat: Treatment)

Envir	Oper	Treat	CRB	STR	FC	HC	Region of RN
NS	Lesion	VHC	ND	10,47 \pm 2,49	0,97 \pm 0,26	ND	0,9 \pm 0,28
NS	Lesion	DMI	ND	10,95 \pm 4,87	1,15 \pm 0,43	ND	1,49 \pm 0,8
NS	Lesion	TRAM	ND	7,23 \pm 1,64	1,2 \pm 0,13	ND	1,45 \pm 1,06
NS	Sham	VHC	ND	8,94 \pm 2,62	0,95 \pm 0,26	ND	0,56 \pm 0,2
NS	Sham	DMI	ND	12,6 \pm 4,84	1,57 \pm 0,65	ND	1,56 \pm 0,31
NS	Sham	TRAM	ND	10,74 \pm 2,01	1,59 \pm 0,50	ND	0,83 \pm 0,25
S	Lesion	VHC	ND	8,85 \pm 4,02	0,8 \pm 0,25	ND	0,96 \pm 0,44
S	Lesion	DMI	ND	7,68 \pm 1,37	1,66 \pm 0,61	ND	0,96 \pm 0,34
S	Lesion	TRAM	ND	11,86 \pm 2,61	1,35 \pm 0,48	ND	1,91 \pm 1,34
S	Sham	VHC	ND	12,8 \pm 5,72	1,54 \pm 0,61	ND	0,64 \pm 0,31
S	Sham	DMI	ND	7,13 \pm 1,96	0,95 \pm 0,37	ND	1,23 \pm 0,73
S	Sham	TRAM	ND	10,25 \pm 3,4	1,02 \pm 0,38	ND	0,92 \pm 0,25

4.3.1.2.6 DOPAC Content

The effects of 5, 7-DHT lesion on the level of DOPAC are presented in Table 18. The level of DOPAC could not be determined in the cerebellum and the hippocampus, because the level of DOPAC was low and highly variable in these regions. We did not observe any significant differences between groups for the DOPAC level in the striatum ($H=13.63$, $P=0.254$), the frontal cortex ($H=5.45$, $P=0.907$) and the region of the raphe nucleus ($H=11.83$, $P=0.376$).

Table 18. Effect of 5, 7-DHT lesion on the DOPAC level in the cerebellum (CRB), striatum (STR), frontal cortex (FC), hippocampus (HC) and the region of the raphe nucleus (RN) in the non-stressed (NS) and stressed (S) mice which were treated either vehicle (VHC) or drugs (tramadol (TRM) 20 mg/kg, i.p. or desipramine (DMI) 10 mg/kg, i.p). The drugs were administered during 4 weeks. All data are expressed in terms of $\mu\text{M/g}$ tissue weight. Data are presented as means \pm S.E.M. ND: Not determined. (Envir: Environment, Oper: Operation, Treat: Treatment)

Envir	Oper	Treat	CRB	STR	FC	HC	Region of RN
NS	Lesion	VHC	ND	6,92 \pm 1,99	0,75 \pm 0,28	ND	0,67 \pm 0,18
NS	Lesion	DMI	ND	6,14 \pm 2	1 \pm 0,35	ND	0,85 \pm 0,43
NS	Lesion	TRAM	ND	6,91 \pm 1,56	0,74 \pm 0,19	ND	0,88 \pm 0,19
NS	Sham	VHC	ND	7,82 \pm 2,22	0,56 \pm 0,14	ND	0,86 \pm 0,22
NS	Sham	DMI	ND	10,58 \pm 2,54	0,87 \pm 0,41	ND	0,99 \pm 0,19
NS	Sham	TRAM	ND	10,44 \pm 1,91	0,72 \pm 0,17	ND	0,69 \pm 0,17
S	Lesion	VHC	ND	3,89 \pm 1,73	0,73 \pm 0,27	ND	0,93 \pm 0,2
S	Lesion	DMI	ND	5,45 \pm 1,41	0,91 \pm 0,28	ND	0,93 \pm 0,17
S	Lesion	TRAM	ND	6,93 \pm 1,73	0,9 \pm 0,27	ND	1,08 \pm 0,2
S	Sham	VHC	ND	4,50 \pm 1,25	0,81 \pm 0,15	ND	0,59 \pm 0,09
S	Sham	DMI	ND	5,74 \pm 1,57	0,61 \pm 0,15	ND	0,79 \pm 0,18
S	Sham	TRAM	ND	9,35 \pm 2,35	1,16 \pm 0,17	ND	1,08 \pm 0,24

4.3.2. The Effects of Pindolol on the Antidepressant-like Effects of Tramadol and Desipramine in the UCMS model in BALB/c Mice

As no effects of the pharmacological treatments were observed in non-stressed mice in the experiment that we searched the effects of tramadol and desipramine, we did not include these non-stressed groups in the experiment, which aimed at investigating the role of 5-HT_{1A} receptors on the actions of desipramine and tramadol.

4.3.2.1. Coat State

The effect of pindolol (10 mg/kg, i.p) on the antidepressant-like actions of tramadol and desipramine over the coat state are shown in Fig.23. This figure illustrates the total score of each week to demonstrate whether pindolol acts on the onset of the antidepressant-like effects of tramadol and desipramine. The test of Kruskal-Wallis H revealed significant differences between all the groups ($H=13.545$, $P=0.019$). In this experimental group, tramadol ($P=0.001$) and desipramine ($P=0.005$) significantly reversed the degradation induced by UCMS in stressed mice when compared to vehicle group. During the drug treatment regimen every week, we observed no effect of pindolol on the onset of the antidepressant-like actions of tramadol and desipramine (P values for tramadol and desipramine combined with pindolol were: after 1 week drug administration $P=0.618$, $P=0.150$, after 2 weeks $P=0.836$, $P=0.188$ and after 3 weeks $P=0.156$, $P=0.522$, respectively when compared to the either tramadol or desipramine treatment). All the groups except pindolol alone ($P=0.181$) group significantly diminished the total score of coat state at the end of the UCMS regimen when compared to the stressed vehicle group (Fig.24; $P= 0.019$).

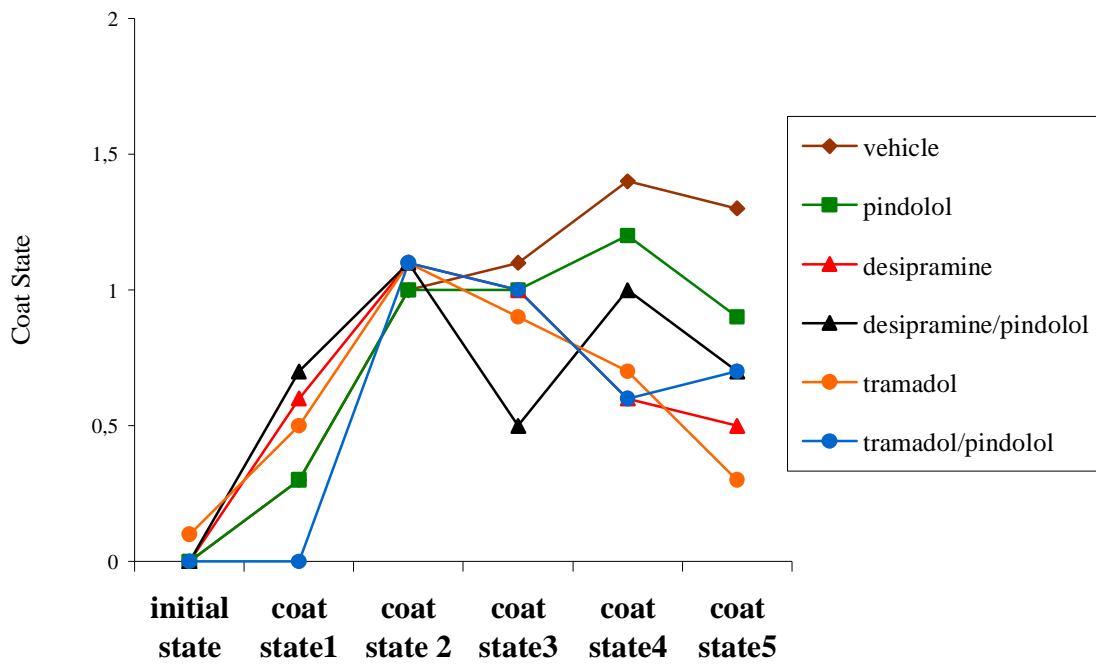


Fig.23. Effect of pindolol (10 mg/kg, i.p) on the antidepressant-like actions of desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) over the coat state in stressed mice during the 4 weeks of treatment. Initial state corresponds to the state of the coat before the UCMS regimen. The standart errors are not indicated in order to clarify the figure.

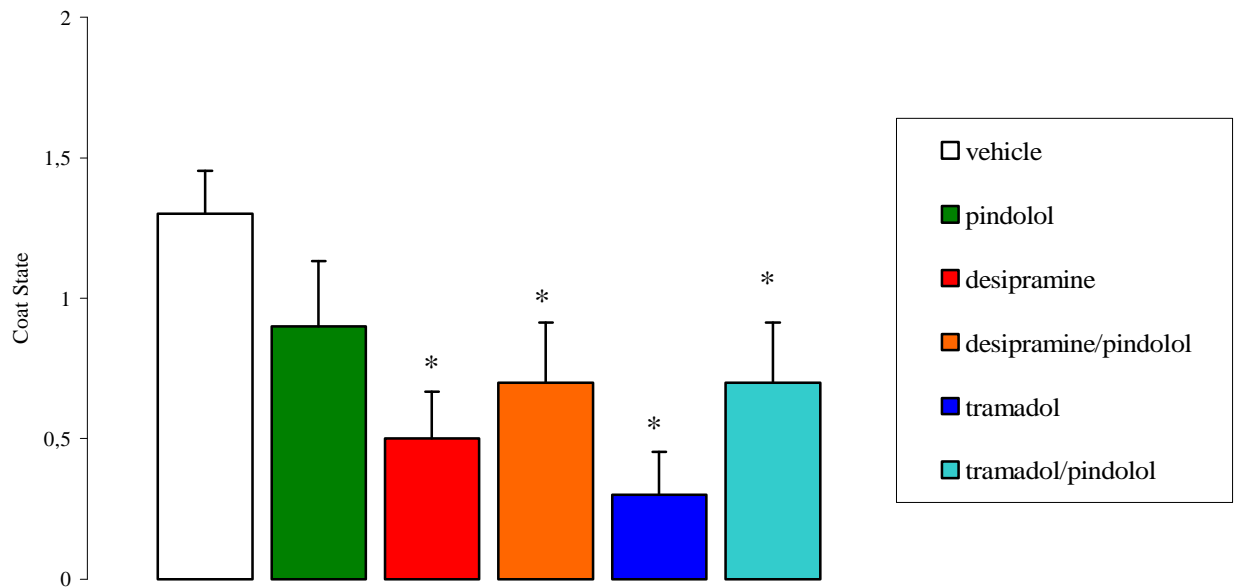


Fig.24. Effect of pindolol (10 mg/kg, i.p) on the antidepressant-like actions of desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) over the coat state in stressed mice after the end of the UCMS regimen. The drugs were administered during 4 weeks. * P<0.05, significantly different when compared to the stressed vehicle.

4.3.2.2. Splash Test

The effect of pindolol on the actions of tramadol and desipramine on the total frequency of the grooming behaviour in the splash test are shown in Fig.25. There was an overall treatment effect ($H=29.382$, $P= 0.000$). Moreover, all of the groups except pindolol/vehicle group significantly augmented the total frequency of the grooming behaviour when compared to the stressed vehicle ($P<0.05$). Pindolol did not modify the effects of tramadol ($P= 0.322$) and desipramine ($P= 0.06$) on this parameter.

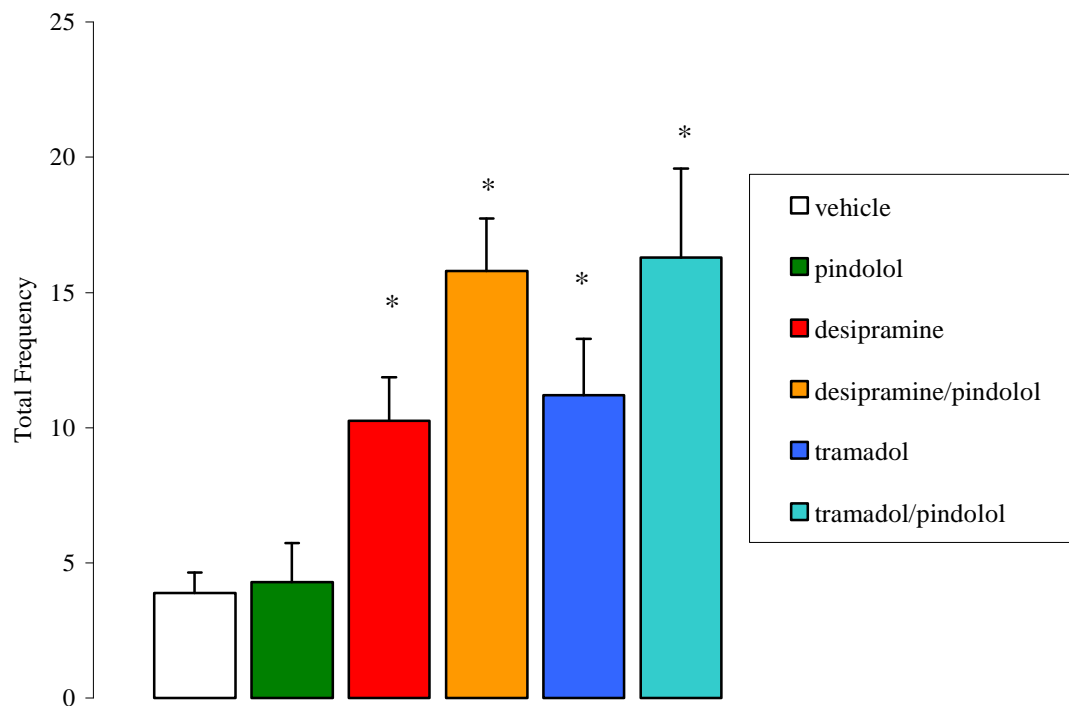


Fig.25. Effect of pindolol (10 mg/kg, i.p) on the antidepressant-like actions of desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) on the total frequency of the grooming behaviour during the splash test. The drugs were administered during 4 weeks. * $P<0.05$, significantly different when compared to the stressed vehicle. Data are means \pm SEM.

4.3.2.3. Body Weight and Locomotor Activity

We did not observe any significant difference between the groups for body weight ($H=8.798$, $P=0.117$) and locomotor activity ($H=6.316$, $P=0.277$). These results are shown in Table 19.

Table 19. The effects of pindolol (10 mg/kg, i.p), tramadol (20 mg/kg, i.p), desipramine (10 mg/kg, i.p) and their mixture on the body weight and locomotor activity in stressed mice. Results are shown as the means \pm S.E.M.

Environment	Treatment	Body Weight	Locomotor Activity
Stressed	Vehicle	30.58 \pm 0.35	2427.70 \pm 309.74
Stressed	Pindolol	31.07 \pm 0.55	2477.90 \pm 317.53
Stressed	Desipramine	30.63 \pm 0.56	2860.90 \pm 257.19
Stressed	Desipramine+Pindolol	29.26 \pm 0.53	3185.20 \pm 346.83
Stressed	Tramadol	29.64 \pm 0.57	2444.50 \pm 412.46
Stressed	Tramadol+Pindolol	29.32 \pm 0.58	2077.70 \pm 328.32

4.4. The Participation of the Noradrenergic System in the Antidepressant-like Effects of Tramadol and Desipramine in the UCMS Model in BALB/c Mice

The last part of this study was undertaken to clarify the contribution of the noradrenergic system especially the β , β_2 and α_2 adrenoreceptors via the non-selective β -adrenoreceptor antagonist propranolol, selective β_2 -adrenoreceptor antagonist ICI 118,551 and α_2 -adrenoreceptor antagonist yohimbine, respectively.

4.4.1. Determination of NA and its Metabolite MHPG Level After the End of the UCMS Model: the Effects of Tramadol and Desipramine

The effects of chronic desipramine and tramadol on the MHPG and NA level in LC, hypothalamus, hippocampus and cerebellum are presented in Table 20. Kruskal-Wallis H revealed significant effects between groups for the level of MHPG ($H=7.775$, $P=0.021$) and NA ($H=8.939$, $P=0.011$) in the LC. MHPG and NA level were enhanced by desipramine ($P=0.021$, $P=0.011$, respectively) and tramadol ($P=0.01$, $P=0.01$, respectively) treatment. Kruskal-Wallis test showed a significant difference between groups for the MHPG level in hypothalamus ($H=12.149$, $P=0.002$), while NA level remained unchanged ($P=0.315$). Indeed, the level of MHPG in this area was significantly augmented by desipramine ($P=0.000$) and tramadol ($P=0.019$). In the hippocampus significant differences among groups appeared ($H=5.661$, $P=0.05$, $H=8.057$, $P=0.01$, respectively for NA and MHPG level). Desipramine significantly increased the NA level ($P=0.04$), while it did not have a significant effect on the level of MHPG ($P=0.122$). Moreover, in this region, tramadol augmented significantly both MHPG ($P=0.008$) and NA ($P=0.05$) level. Neither MHPG level nor NA level were modified in cerebellum ($H=3.109$, $P=0.211$, $H=1.220$, $P=0.543$, respectively).

Table 20. Effect of treatment with desipramine (10 mg/kg, i.p) and tramadol (20 mg/kg, i.p) on the level of MHPG and NA in the LC, hypothalamus, hippocampus and cerebellum in the stressed mice. The drugs were administered during 4 weeks. All data are expressed in terms of $\mu\text{M/g}$ tissue weight. Data are presented as means \pm S.E.M. * $P < 0.05$, significantly different when compared to the stressed vehicle.

Locus Coeruleus	MHPG ($\mu\text{M/g}$ tissue)	NA ($\mu\text{M/g}$ tissue)
Vehicle	1,89 \pm 0,63	2,33 \pm 0,17
Desipramine	6,28 \pm 2,01*	3,22 \pm 0,29*
Tramadol	5,72 \pm 1,04*	4,07 \pm 0,54*
Hypothalamus	MHPG ($\mu\text{M/g}$ tissue)	NA ($\mu\text{M/g}$ tissue)
Vehicle	1,37 \pm 0,21	2,35 \pm 0,20
Desipramine	5,68 \pm 1,21*	3,13 \pm 0,88
Tramadol	5,04 \pm 1,60*	3,33 \pm 0,52
Hippocampus	MHPG ($\mu\text{M/g}$ tissue)	NA ($\mu\text{M/g}$ tissue)
Vehicle	2,12 \pm 0,33	1,18 \pm 0,25
Desipramine	3,23 \pm 0,40	1,82 \pm 0,38*
Tramadol	5,63 \pm 1,13*	1,67 \pm 0,16*
Cerebellum	MHPG ($\mu\text{M/g}$ tissue)	NA ($\mu\text{M/g}$ tissue)
Vehicle	2,85 \pm 0,54	0,14 \pm 0,075
Desipramine	4,74 \pm 0,74	0,36 \pm 0,15
Tramadol	3,80 \pm 0,53	1,12 \pm 0,96

4.4.2. The Effects of Propranolol, ICI 118,551 and Yohimbine on the Antidepressant-like Effects of Tramadol and Desipramine in the UCMS Model in BALB/c Mice.

4.4.2.1. The Effects of Propranolol on the Antidepressant-like Effects of Tramadol and Desipramine in the UCMS Model in BALB/c Mice

4.4.2.1.1. Coat State

The effects of propranolol on the actions of tramadol and desipramine are shown in Fig.26. Analysis of the total score of the coat state by the test of Kruskal-Wallis H revealed significant difference between the groups ($H=82.571$, $P=0.000$). A comparison between the total score of the non-stressed vehicle and stressed vehicle groups showed that UCMS induced a significant degradation of the coat state ($P=0.000$). This effect was significantly improved by the treatment with tramadol (20 mg/kg, $P=0.001$) and desipramine (10 mg/kg, $P=0.011$) in stressed mice (Fig.26). No treatment elicited any effect in non-stressed mice ($P=0.939$ for desipramine and $P=0.292$ for tramadol).

Moreover the antidepressant-like effects of tramadol and desipramine were significantly antagonized by propranolol (5 mg/kg, i.p) (P values for desipramine and tramadol combined with propranolol when compared to desipramine or tramadol alone, $P=0.028$, $P=0.000$, respectively). Propranolol (5 mg/kg) had no effect on the total score of the coat state ($P=0.693$) neither in stressed mice nor non stressed mice ($P=0.939$) when given alone.

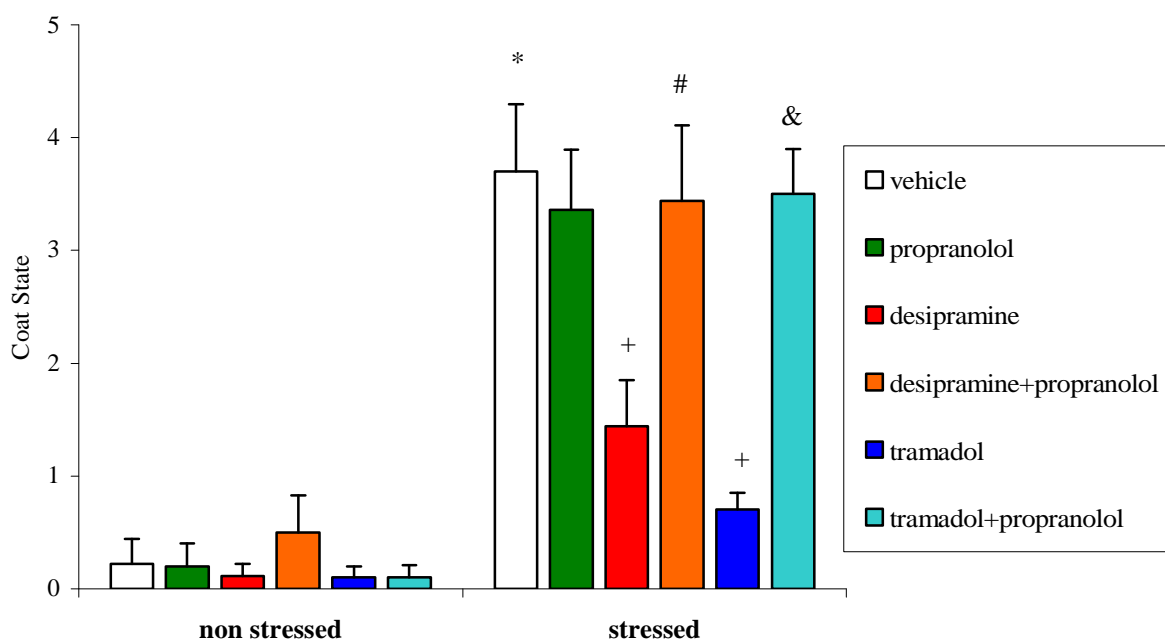


Fig.26. Effect of propranolol (5 mg/kg, i.p) on the antidepressant-like actions of desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) on the coat state in stressed and non-stressed mice after the end of the UCMS regimen. The drugs were administered during 4 weeks. * $P < 0.05$, significantly different when compared to the non-stressed vehicle, + $P < 0.05$, significantly different when compared to the stressed vehicle, # $P < 0.05$, significantly different when compared to the stressed desipramine, & $P < 0.05$, significantly different when compared to the stressed tramadol group. Data are presented as means \pm S.E.M.

4.4.2.1.2. Splash Test

The effects of tramadol and desipramine on the grooming frequency are shown in Fig.27. By the Kruskal-Wallis H test; we observed a significant difference among groups ($H=36.897$, $P=0.000$). Mice from the non-stressed vehicle group groomed significantly more than mice from the stressed vehicle group ($P=0.016$). Neither tramadol nor desipramine changed the grooming frequency in the non-stressed mice ($P=0.672$, $P=0.353$, respectively). Both tramadol ($P=0.004$) and desipramine ($P=0.001$) significantly augmented the frequency of the grooming behaviour in the stressed mice in the splash test and these effects were blocked by propranolol (P values for desipramine and tramadol combined with propranolol compared to desipramine and tramadol; $P=0.004$, $P=0.03$, respectively). However, propranolol did not show any

effect on the grooming behaviour in the splash test when compared to vehicle in non-stressed ($P=0.205$) and in stressed ($P=0.970$) mice.

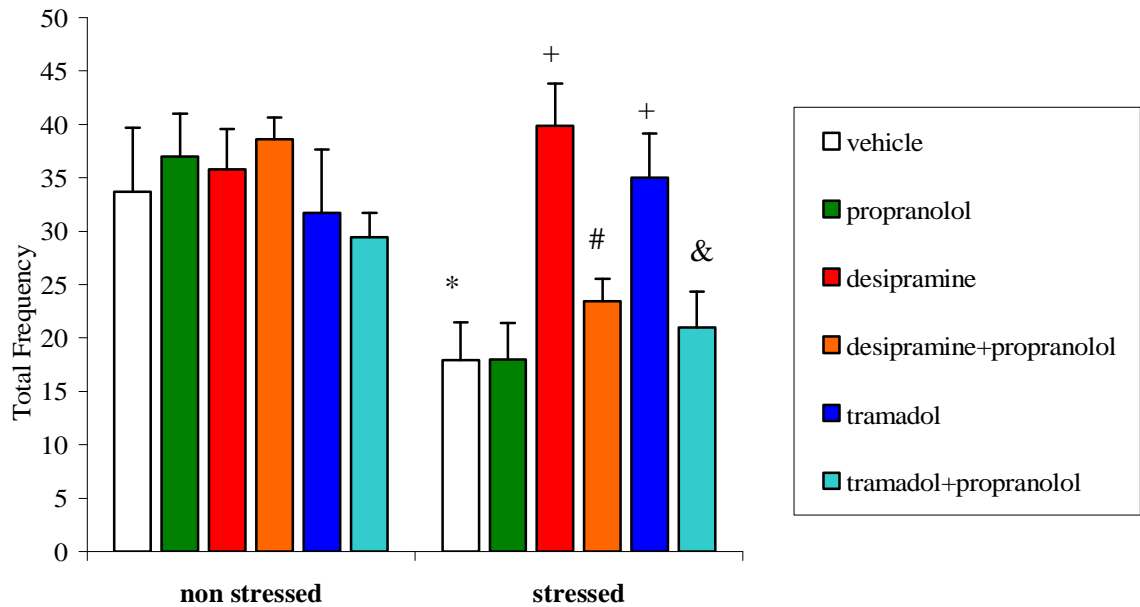


Fig.27. Effect of propranolol (5 mg/kg, i.p) on the antidepressant-like actions of desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) on the total frequency of the grooming behaviour in non-stressed and stressed mice during the splash test. The drugs were administered during 4 weeks. * $P<0.05$, significantly different when compared to the non-stressed vehicle, + $P<0.05$, significantly different when compared to the stressed vehicle, # $P<0.05$, significantly different when compared to the stressed desipramine, & $P<0.05$, significantly different when compared to the stressed tramadol group. Data are presented as means \pm S.E.M.

4.4.2.1.3. Body Weight and Locomotor Activity

Along the UCMS regimen, no significant difference between groups ($H=18.54$ $P=0.070$) could be shown for the body weight. Further, no significant impairment of locomotor activity due to UCMS regimen or treatment was observed ($H=13.02$, $P=0.292$) (Table 21).

Table 21. The effects of propranolol (5 mg/kg, i.p) tramadol (20 mg/kg, i.p), desipramine (10 mg/kg, i.p) and their mixture on the body weight and locomotor activity in non-stressed and stressed mice. The drugs were administered during 4 weeks. Results are shown as the means \pm S.E.M.

Environment	Treatment	Body Weight	Locomotor Activity
Non-stressed	Vehicle	32.71 \pm 1.91	930.2 \pm 144.23
Non-stressed	Propranolol	32.59 \pm 1.79	949.87 \pm 110.36
Non-stressed	Desipramine	32.84 \pm 0.94	964.12 \pm 155.79
Non-stressed	Desipramine+Propranolol	29.57 \pm 2.24	776 \pm 163.41
Non-stressed	Tramadol	28.6 \pm 1.53	1186.5 \pm 130.53
Non-stressed	Tramadol+Propranolol	28.45 \pm 1.35	900.4 \pm 237.98
Stressed	Vehicle	33.93 \pm 1	1082.66 \pm 146.36
Stressed	Propranolol	33.67 \pm 1.39	1022.09 \pm 149.42
Stressed	Desipramine	31.61 \pm 1.38	953.11 \pm 155.79
Stressed	Desipramine+Propranolol	31.47 \pm 1.26	984.5 \pm 159.08
Stressed	Tramadol	28.76 \pm 1.43	1225.22 \pm 227.36
Stressed	Tramadol+Propranolol	32.46 \pm 0.76	1515.2 \pm 154.41

4.4.2.2. Effect of ICI 118,551 on the Antidepressant-like Action of Desipramine and Tramadol

As no effects of the pharmacological treatments were observed in non-stressed mice, we did not include these non-stressed groups in the experiment that searched the effects of selective β_2 -adrenergic receptor antagonist ICI 118,551 on the antidepressant-like effects of desipramine and tramadol in stressed mice.

4.4.2.2.1. Coat State

The effect of ICI 118,551 (2 mg/kg, i.p) on the antidepressant-like action of desipramine and tramadol on the coat state are shown in Fig.28. We observed a significant difference between groups ($H=28.193$, $P=0.000$). Tramadol ($P=0.004$) and desipramine ($P=0.000$) significantly reversed the degradation of the coat state induced by UCMS. The antidepressant-like effect of both drugs was significantly antagonized by ICI 118,551 (P values for desipramine and tramadol combined with ICI 118,551

when compared to desipramine or tramadol treated mice; $P=0.001$ and $P=0.009$, respectively). ICI 118,551 alone did not elicit any effect on the total score of the coat state ($P=0.409$).

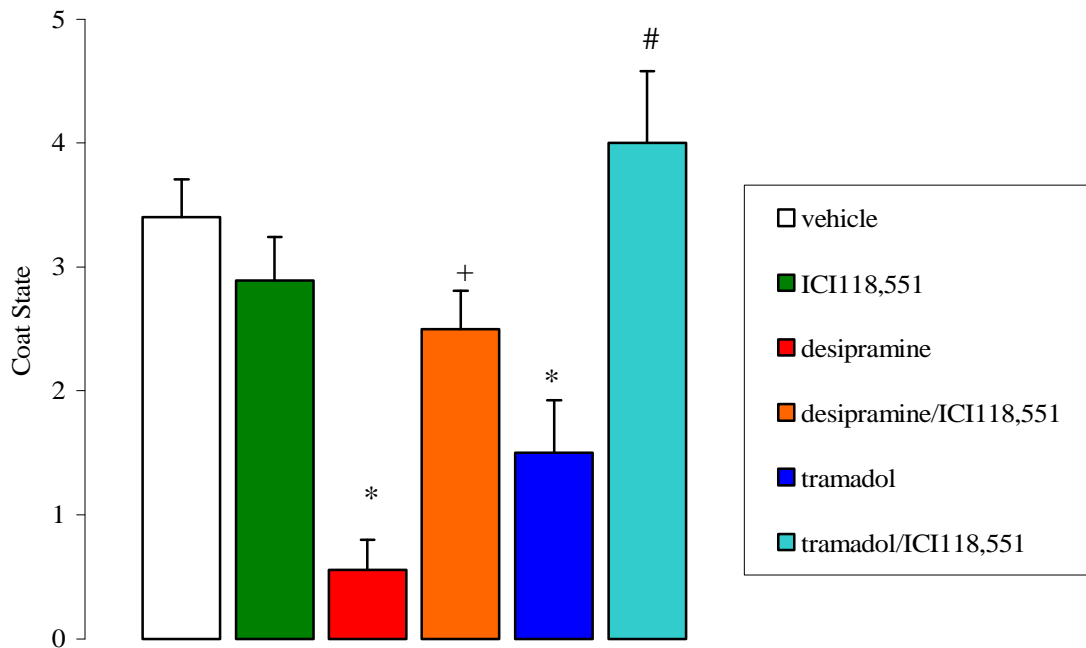


Fig.28. Effect of ICI 118,551 (2 mg/kg, i.p) on the antidepressant-like actions of desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) on the coat state in stressed mice after the end of the UCMS regimen. The drugs were administered during 4 weeks. * $P<0.05$, significantly different when compared to the vehicle, + $P<0.05$, significantly different when compared to the stressed desipramine, # $P<0.05$, significantly different when compared to the stressed tramadol group. Results are presented as means \pm S.E.M.

4.4.2.2.2. Splash Test

The antagonism by ICI 118,551 of the antidepressant-like effects of tramadol and desipramine in the splash test are shown in Fig.29. There was an overall treatment effect ($H=17.026$, $P=0.004$). Desipramine ($P=0.010$) and tramadol ($P=0.016$) increased the total frequency of the grooming behaviour and these effects were significantly blocked by ICI 118,551 (P values for desipramine and tramadol combined with ICI 118,551 when compared to desipramine or tramadol treated mice; $P=0.006$, $P=0.03$

respectively). ICI 118,551 had no effect on the total frequency of the grooming behaviour compared to vehicle when given alone ($P=0.838$).

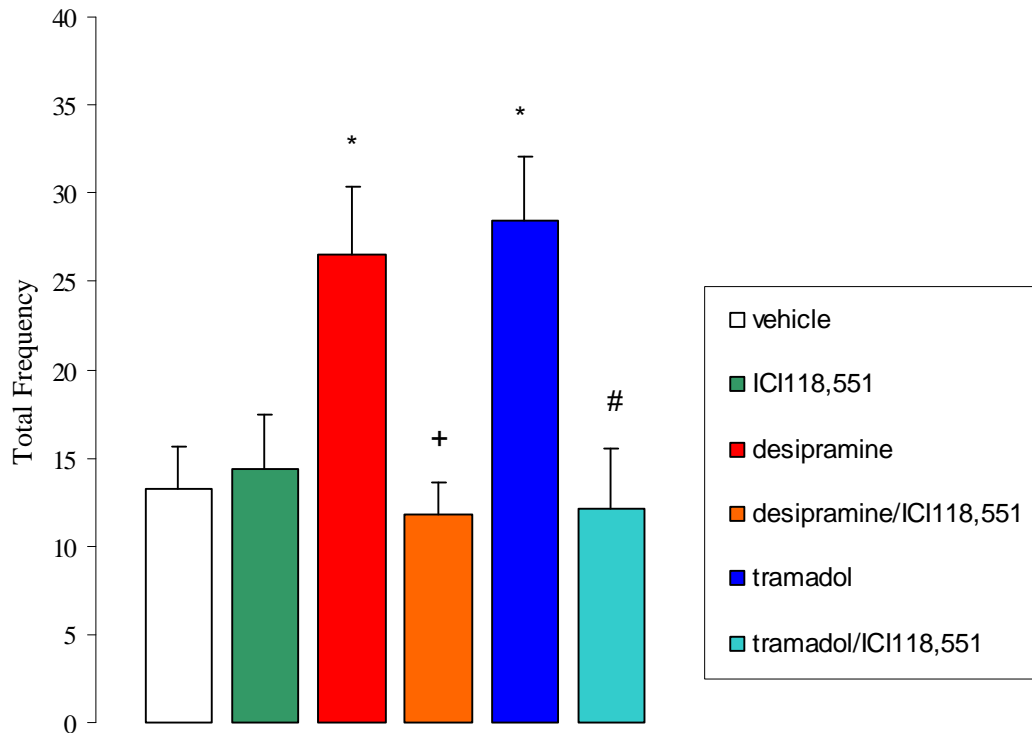


Fig.29. Effect of ICI 118,551 (2 mg/kg, i.p) on the antidepressant-like actions of desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) on the total frequency of the grooming behaviour during the splash test. The drugs were administered during 4 weeks. * $P<0.05$, significantly different when compared to the stressed vehicle, + $P<0.05$, significantly different when compared to the stressed desipramine, # $P<0.05$, significantly different when compared to the stressed tramadol group. Data are presented as means \pm S.E.M.

4.4.2.2.3. Body Weight and Locomotor Activity

We did not observe any significant difference between the groups for body weight ($H=10.16$, $P=0.071$) and locomotor activity ($H=10.44$, $P=0.064$) (Table 22).

Table 22. The effects of ICI 118,551 (2 mg/kg, i.p) tramadol (20 mg/kg, i.p), desipramine (10 mg/kg, i.p) and their mixture on the body weight and locomotor activity in stressed mice. All the drugs were administered during 4 weeks. Results are shown as the means \pm S.E.M.

Environment	Treatment	Body Weight	Locomotor Activity
Stressed	Vehicle	29.23 \pm 0.39	1816.55 \pm 151.21
Stressed	ICI 118,551	29.59 \pm 0.39	1711.9 \pm 160.33
Stressed	Desipramine	29.25 \pm 0.73	1865.22 \pm 92.6
Stressed	Desipramine+ICI 118,551	29.05 \pm 0.38	1567.28 \pm 58.30
Stressed	Tramadol	27.99 \pm 0.25	1425.66 \pm 88.30
Stressed	Tramadol+ICI 118,551	28.08 \pm 0.42	1943.9 \pm 118.37

4.4.2.3. Effect of Yohimbine on the Antidepressant-like Action of Desipramine and Tramadol

4.4.2.3.1. Coat State

The effect of α_2 -adrenoreceptor antagonist yohimbine (2 mg/kg, i.p) on the antidepressant-like actions of tramadol and desipramine over the coat state are presented in Fig.30. We observed a significant difference between the groups ($H=21.019$, $P=0.001$). Both tramadol ($P=0.003$) and desipramine ($P=0.017$) treatment induced a significant improvement of the coat state of the mice when compared to stressed vehicle group. The antidepressant-like effects of both drugs were significantly antagonized by yohimbine (P values for desipramine and tramadol combined with yohimbine when compared to desipramine or tramadol treated mice; $P=0.009$ and $P=0.005$, respectively).

However, yohimbine had no effect on the total score of the coat state compared to vehicle, when given alone ($P= 0.240$).

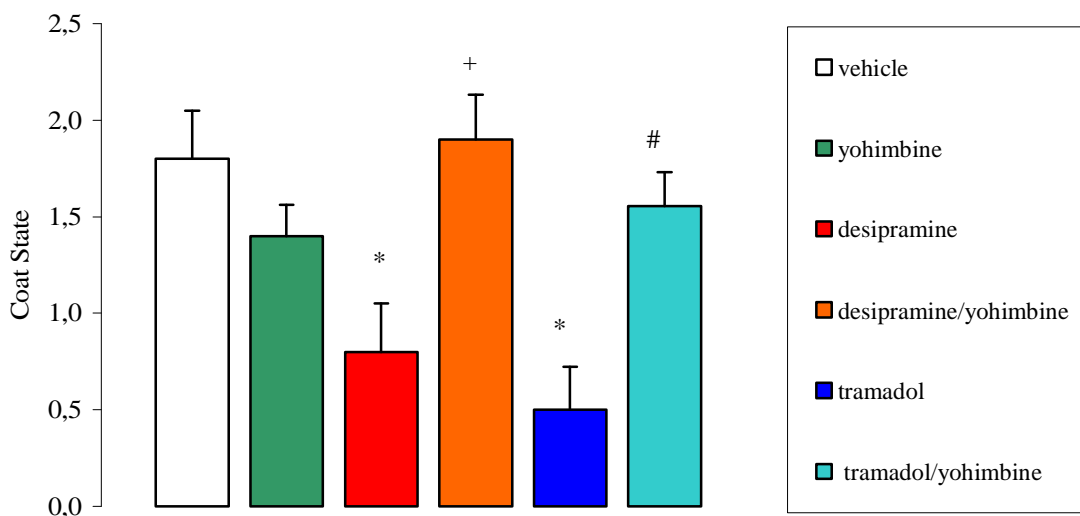


Fig.30. Effect of yohimbine (2 mg/kg, i.p) on the antidepressant-like actions of desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) over the coat state in stressed mice. The drugs were administered during 4 weeks. * $P < 0.05$, significantly different when compared to the stressed vehicle, + $P < 0.05$, significantly different when compared to the stressed desipramine, # $P < 0.05$, significantly different when compared to the stressed tramadol group. Results are shown as the means \pm S.E.M.

4.4.2.3.2. Splash Test

The antagonism by yohimbine of the antidepressant-like effects of tramadol and desipramine in the splash test are shown in Fig.31. There was an overall treatment effect ($H=16.669$, $P=0.05$). Desipramine and tramadol increased grooming behaviour ($P=0.017$ and $P=0.002$, respectively) and these effects were blocked by yohimbine (P values for desipramine and tramadol combined with yohimbine when compared to desipramine or tramadol $P=0.009$, $P=0.005$, respectively). Yohimbine also did not elicit any effect compared to vehicle, when given alone ($P=0.448$).

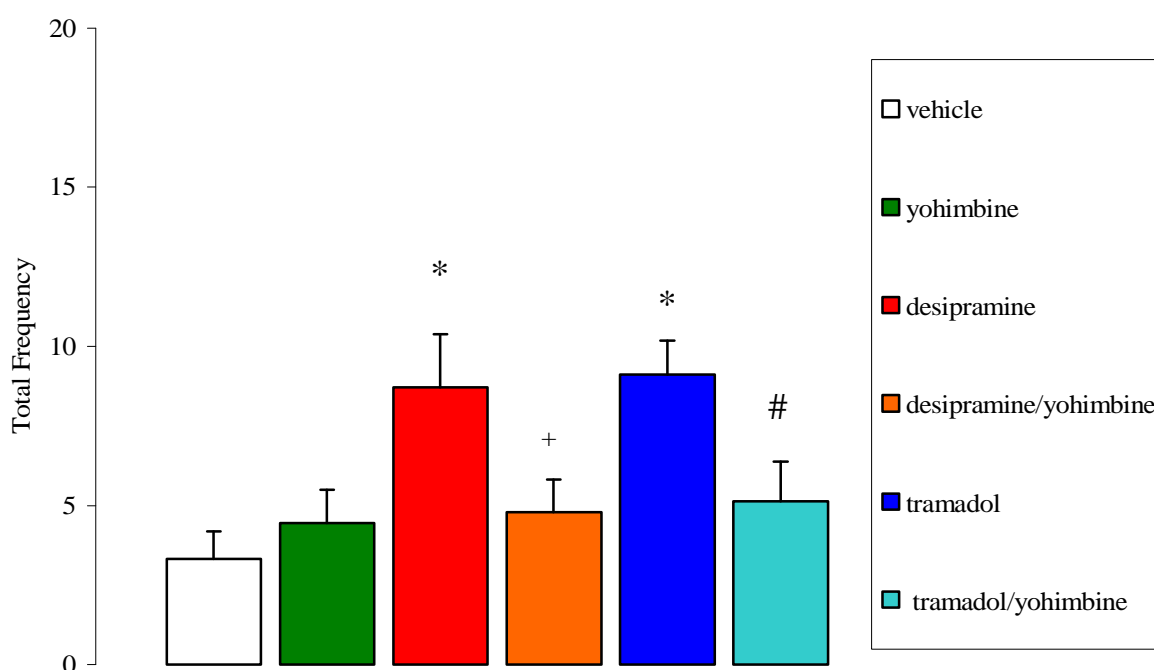


Fig.31. Effect of yohimbine (2 mg/kg, i.p) on the antidepressant-like actions of desipramine (10 mg/kg, i.p) and tramadol (20mg/kg, i.p) on the total frequency of the grooming behaviour during the splash test. The drugs were administered during 4 weeks. * $P < 0.05$, significantly different when compared to the stressed vehicle, + $P < 0.05$, significantly different when compared to the stressed desipramine, # $P < 0.05$, significantly different when compared to the stressed tramadol group.

4.4.2.3.3. Body Weight and Locomotor Activity

Body weight of the mice didn't show significant difference between groups ($H = 2.38$, $P = 0.793$) and also no significant impairment of locomotor activity due to treatment was observed ($H = 4.325$, $P = 0.504$). These results are shown in Table 23.

Table 23. The effects of yohimbine (2 mg/kg, i.p) tramadol (20 mg/kg, i.p), desipramine (10 mg/kg, i.p) and their mixture on the body weight and locomotor activity in stressed mice. Results are shown as the means \pm S.E.M.

Environment	Treatment	Body Weight	Locomotor Activity
Stressed	Vehicle	31.10 \pm 0.44	2308.70 \pm 346.92
Stressed	Yohimbine	30.90 \pm 0.43	1847.70 \pm 244.62
Stressed	Desipramine	30.50 \pm 0.45	2435.75 \pm 348.61
Stressed	Desipramine+Yohimbine	31.05 \pm 0.47	2056.70 \pm 260.18
Stressed	Tramadol	30.95 \pm 0.31	2765.11 \pm 284.25
Stressed	Tramadol+Yohimbine	31.05 \pm 0.47	2151.25 \pm 408.28

5. DISCUSSION

5.1. The Effects of Strain Differences and Validation of Some Antidepressants in the UCMS

In almost all of the behavioural models used in depression research, substantial strain differences have been observed. The analyses of phenotypic behavioural differences in both inbred and outbred strains may point to an underlying genetic basis of a given behavioural trait relevant to depression and antidepressant action. Recent studies in various animal models of antidepressant-action suggest that multi-strain comparisons may be needed to prevent false negative screening of compounds in a given paradigm⁹². Besides, recent papers that question the validity of UCMS model focused on the different results obtained from different laboratories^{11,94}.

For these reasons, to shed light on the potential genetic contribution, we examined potential differences between one outbred and one inbred strain in response to pharmacologically different types of antidepressants in the UCMS model. The present data clearly show marked differences between the strains of mice in their sensitivity to the UCMS regimen. UCMS-induced coat state deterioration was observed in BALB/c mice but not in Swiss mice. The results obtained in the present experiment are consistent with previous studies reporting that stressed BALB/c mice displayed higher coat state degradation than non-stressed BALB/c after 2 week of subchronic stress period²⁶⁶. On the other hand, it is well known that Swiss mice are an outbred strain and genetic contributions are more variables for outbred than for inbred strain. Lucki et al.²⁶⁷ have reported that the outbred strains such as CD-1, CF-1, Swiss and Webster show more variability in their performances in the FST than inbred.

Furthermore, we observed significant differences between non-stressed and stressed vehicle group in the splash test in BALB/c mice but not in Swiss strain. Non-stressed mice groomed more than stressed mice in BALB/c mice. These results have been considered analogous to the observation that depressed patients execute with great effort even the smallest tasks, often leading to poor hygiene⁹⁴.

According to these findings, it is possible to suggest that the Swiss strain appears to be much more resistant to the effects of UCMS than the BALB/c mice.

Regarding the treatment, all of the antidepressants that we tested significantly improved the deterioration of the coat induced by UCMS in BALB/c mice but not in Swiss mice. Fluoxetine, desipramine and imipramine augmented the grooming behaviour in stressed mice when compared to the stressed vehicle group in BALB/c mice, while maprotiline did not show any significant effect in the splash test.

Nevertheless, none of these drugs induced any effect on the grooming behaviour when compared to stressed vehicle in Swiss mice in the splash test. But, it is evident that the antidepressants used in this study did not manage to show any effects in Swiss mice because the UCMS regimen did not induce enough changes neither on the state of the coat nor in the splash test. Indeed, it has already been shown that the antidepressants are devoid of effects in non-stressed mice¹⁰⁵ or normal subjects (non-stressed patients)²⁶⁸. On the other hand, the lack of antidepressant action cannot be related with their proper effects. In fact, it has been published that the Swiss strain was sensitive to imipramine and paroxetine^{269, 270} in the TST. In addition, fluoxetine reduced immobility time in BALB/cJ and Swiss mice in the FST²⁶⁷.

Two different mechanisms can be considered to explain the different response to antidepressants among strains, including pharmacodynamic or pharmacokinetic parameters. Thus, it is well known that strains of mice may differ in their sensitivity to a drug with variations in absorption, distribution and metabolism²⁷¹. Alternatively, these differential responses to antidepressants could depend on differences in receptor activities^{272, 273}. For example, in the hippocampus and LC of C₅₇BL, the 5-HT synthesis and release are higher than in the BALB/c strain but in the caudate nucleus the initial accumulation of tryptophan is similar in both strains²⁷⁴. These differences disappear when the study is carried on the raphe dorsalis and the raphe centralis nuclei²⁷⁵. However, there is no recent evidence to clarify these hypotheses beyond these two strains.

On the other hand, no differences between body weight was observed in both strains. A recent study has reported that on eight strains of mice, six strains (FVB/NA, BALB/cByJ, DBA/2J, 129SvJ, C3H/HeJ and BA) showed a physical state deterioration induced by UCMS, and from these six strains, only two of them (129 SvJ and C3H/HeJ) showed normal body weight gain during UCMS regimen whereas stress procedure diminished the body weight of the other strains²⁵⁹.

It has been reported that BALB/c BJ mice exhibited the greatest level of activity and showed more anxiety-like behaviours than C₅₇BL/6J and DBA/2J²⁷⁶. In the present study, neither the regimen of UCMS nor the antidepressant treatments changed the locomotor activity in both strains. In addition, our results showed that BALB/c BJ mice were more active than the Swiss mice.

Taken together, the study presented here and those reported earlier with BALB/c mice suggest that BALB/c is a sensitive strain to the physiological and behavioural effects of UCMS.

In the light of these results, we decided to use BALB/c mice for the rest of the studies in the present Thesis. We also chose desipramine as a positive control as we wanted to use an antidepressant which selectively acts on one monoaminergic system. Indeed, desipramine is a selective NA reuptake inhibitor and it is active in the UCMS model as we showed in this part.

5.2. The Effects of Tramadol and Desipramine in an UCMS Model in BALB/c Mice

In the second part of the study, the antidepressant-like effects of tramadol and desipramine were investigated in the UCMS model in BALB/c mice. Analysis of data showed that the UCMS regimen induced degradation in the state of the coat and decreased the grooming behaviour in the splash test in the stressed/vehicle mice when compared to the non-stressed/vehicle mice. Results that we obtained from BALB/c mice are in line with the first part of the study where we showed a deterioration of the coat condition related with stress-induced deficits in grooming, in an antidepressant reversible manner. These alterations cannot be due to trivial effects of the treatments on activity since locomotion in mice remains unchanged. These physical and behavioural abnormalities were counteracted by the chronic treatment of 10 mg/kg desipramine and 20 mg/kg tramadol. These results indicate that desipramine and tramadol elicit antidepressant-like effects in the UCMS. Indeed, it has been already reported for fluoxetine, CRF antagonists and vasopressin V_{1B} receptor antagonists^{33, 41, 124} that they counteracted the effects of chronic stress on the state of the coat and in the splash test. In addition, this result is concordant with the findings obtained from our first part of the experiments and the other's study showing that desipramine increases the grooming behaviour in the UCMS model²⁵¹. Our results also confirm previous studies showing that tramadol has potential antidepressant-like effects in rodents^{6, 7}. Interestingly, these effects only appeared after a chronic treatment: indeed, no effect of these treatments was detected after one or two weeks of the antidepressant administration. Harkin et al.²⁷⁷ showed that the antidepressant effect observed was dependent upon repeated administration over a 4 week period and could not be attributed to any acute interaction between UCMS and drug treatment. Moreau et al.¹⁰⁹ and Valverde et al.²⁷⁸ have also reported that the effects of UCMS can be blocked or reversed by chronic but not acute antidepressant treatment. These data further confirm the predictive validity of the UCMS model.

Neither the UCMS regimen nor the drug treatments changed the locomotor activity. In contrast, Henn and Vollmayr.⁹³ showed that 4 weeks of chronic stress, consisting of intermittent exposure to a rat, intermittent stress and tail suspension, increased locomotor activity and induced loss of body weight. In line with our results,

Liang et al.²⁷⁹ showed that neither single administration nor chronic administration of tramadol induced any effect on locomotor activity in mice.

However, even if UCMS had not effect on body weight, the treatment with tramadol or desipramine diminished it. Lydiard et al.²⁸⁰ reported that the patients receiving desipramine during 12 weeks showed significant weight loss. In addition desipramine treatment had caused decreased food intake and weight loss when administered chronically in rats²⁸¹. In contrast, Shioiri et al.²⁸² showed that desipramine exposure did not seem to have the side effects of weight changes. Thus, the results obtained with desipramine are discrepancies. Besides, to our knowledge, there is no study about the effects of tramadol on the body weight.

Cognitive deficits have been frequently reported in depression²⁸³ and antidepressant therapy may improve cognitive function^{284, 285}. So, we searched the effects of UCMS on the spatial memory using the Morris water maze. Spatial learning was not altered by UCMS in BALB/c mice. The time to find the platform decreased from the first to the last session for every group. In the probe test, no significant differences could be observed between groups. In contrast, Song et al.¹¹² reported that spatial learning of stressed mice after learned helplessness and CMS was significantly lower than those seen in the control group and the mice subjected to these depression models required a longer time to locate the hidden platform than the non-stressed control mice during the learning trials. On the other hand, spatial learning in a water maze was not disturbed by UCMS in C57BL/6 and DBA/2 mice²⁸⁶. So, it is possible that the performance in the water maze depends on strain differences and BALB/c mice may not be sensitive to this task.

Taken together all these data, we can conclude from this section that tramadol and desipramine have antidepressant-like effects in the UCMS model.

5.3. The Contribution of the Serotonergic System to the Antidepressant-like Effects of Tramadol and Desipramine in the UCMS Model in BALB/c Mice

5-HT has been strongly implicated in the etiology of depression and the mechanism of action of antidepressants²⁸⁷. In this part of the study, we were interested in the participation of the serotonergic system in the antidepressant-like effects of tramadol and desipramine.

5.3.1. The Effect of Administration of 5, 7- Dihydroxytryptamine (5, 7-DHT) into the DRN on the Antidepressant-like Effects of Desipramine and Tramadol in the UCMS Model

Local lesions in the CNS are a common tool to interfere with brain structures and neurotransmitter systems, respectively, to assess their relevance for the behaviour and basal brain functions²⁸⁸. To change 5-HT levels in a very early stage of the synthesis, the neurotoxin 5, 7-DHT were used to destroy 5-HT selectively neurons in the raphe nucleus. However, there are two main regions of the raphe nuclei, DRN and MRN. The innervations from the DRN are generally spread out wider over the different parts of the brain when compared to the innervations pattern of the MRN¹⁶². For this reason, in this study the DRN was chosen to inject 5, 7-DHT, thereby destroying 5-HT-containing neurons in this region.

In this study, the administration of 5, 7-DHT into the DRN did not change the coat state or grooming behaviour in the splash test neither in non-stressed mice nor in stressed mice when compared to their sham group. Both in lesioned and sham mice, the UCMS regimen induced degradation in the coat state and decreased the grooming behaviour in the splash test. While the desipramine or tramadol treatment reversed these effects in sham mice, they failed to prevent these abnormalities in lesioned mice.

These results show that the decrements of 5-HT level could not induce depression without a stress. If this was the case, the non-stressed lesioned mice should have symptoms of depression such as degradation in the state of the coat or decreased grooming in the splash test.

On the other hand, the antidepressant-like effects of tramadol were antagonized by the 5, 7-DHT lesion. So it is possible to suggest that tramadol shows its

antidepressant-like effects via the serotonergic system. Indeed, Hopwood et al.²³⁹ reported that tramadol decreases the binding of 5-HT_{2A} receptors and Berrocoso et al.¹⁰ showed that 5-HT_{1A} antagonist WAY 100635 decreased the antidepressant-like effects of tramadol in FST. Surprisingly, the antidepressant-like effects of desipramine were also prevented by the 5, 7-DHT lesion. Thus, it can be speculated that desipramine has an effect on the serotonergic system. Indeed, the 5-HT_{1A} receptor agonist 8-OH-DPAT induced significant anti-immobility effects with subactive doses of desipramine in the FST whereas 5-HT_{1A} receptor antagonist NAN 190, attenuated the anti-immobility effects of desipramine. In contrast, pretreatment with the 5-HT_{1A} antagonist pindolol failed to potentiate the effects of subactive doses of desipramine²⁰². It is important to mention that 8-OH-DPAT acts post-synaptically²⁸⁹ whereas pindolol elicits its effect generally at presynaptic 5-HT_{1A} receptors²⁶⁷. So it is possible to suggest that the action of desipramine seems to be mediated by post-synaptic 5-HT_{1A} receptors. There can be another hypothesis that we can propose to explain the lack of effects of desipramine in the lesioned mice. As we will discuss detailed below, the stress exposure diminished the level of both NA and 5-HT and additionally 5, 7-DHT lesion reduced the 5-HT level. So, it is possible that the augmentation of the NA level induced by desipramine in lesioned stressed mice could not be sufficient to counteract the effects of 5, 7-DHT lesion on the 5-HT level plus the effects of UCMS regimen on the both 5-HT and NA level.

We also investigated the effects of 5, 7-DHT lesion, UCMS regimen and drug treatment on the agonistic behaviour. For this purpose, the attack latency was determined in the resident-intruder test. We showed that the attack latency was diminished by the UCMS exposure in the resident-intruder test. These results are accordance with the study of Mineur et al.²⁶² that reported a clear increase in levels of aggressiveness after UCMS. Moreover, tramadol and desipramine exposure significantly augmented the attack latency both in sham and lesioned stressed mice. In line with our results, depressed patients as well as UCMS-treated mice also exhibit disturbances in 5-HT levels²⁹⁰ and treatment with SSRIs decrease the levels of aggression in humans and in rodents²⁹¹. These effects cannot be dependent on the sedative effects of drugs. Indeed, there are no significant differences for the locomotor activity between the drug treated and vehicle groups as we will point out below. In the

resident intruder test, we also showed that the administration of 5, 7-DHT lesion into the DRN did not change aggressive behaviour in both non-stressed and stressed mice. Likewise, Van der et al.²⁹² have also demonstrated that lesioning 5-HT terminals by 5, 7-DHT clearly decreased the 5-HT and 5-HIAA levels in CSF, but aggressive behaviour remained unaltered in the resident-intruder test.

Furthermore, we did not observe any significant difference between the lesioned group and the sham group in the locomotor activity in non-stressed and stressed mice. In addition, Koprowska and Romaniuk.²⁹³ reported that after the administration of 5, 7-DHT into the DRN, the locomotor activity of cats was not subjected to significant changes. However, Chia et al.²⁹⁴ showed that the intracerebroventricular (i.c.v) administration of 5, 7 DHT mediated decrements of 5-HT, which were accompanied by a marked decline of locomotor activity in C57BL/6. But, it must be noticed that in that study the strain used and the route of administration of 5, 7-DHT are different from our study. On the other hand, the administration of 5, 7-DHT into the DRN did not induce any body weight changes neither in non-stressed nor stressed mice. I.c.v administration of 5, 7-DHT induce body weight loss during 14 days and potentiated the ability of fluoxetine to further inhibit body weight gain²⁹⁵. Curiously, in this part of the study, tramadol and desipramine did not change the body weight.

In order to relate the behavioural effects of 5, 7-DHT administration into DRN with the level of neurotransmitter in target brain regions, we determined the concentrations of monoamines (NA, DA, 5-HT) and of their metabolites (5-HIAA, DOPAC, HVA) in the key areas (striatum, hippocampus, region of the raphe magnus, frontal cortex and cerebellum) forming the neuronal systems, which participate in the central regulation of mood disorders. The animals were treated with desipramine before the 5, 7-DHT injection for minimize the effect of the neurotoxin on the noradrenergic cells. As expected the 5, 7-DHT mediated decrements of the 5-HT level in the striatum, the frontal cortex, the hippocampus and of the 5-HIAA level in the striatum and the hippocampus. In the raphe magnus, 5, 7-DHT also decreased the level of 5-HT and 5-HIAA level but it was not statistically significant. It is possible that the effects of DRN lesion on the 5-HT level in the raphe magnus were compensated by the activity of the MRN on the level of this neurotransmitter.

Descarries et al.²⁹⁶ reported that 70% of the total number of cells in the rat DRN is not 5-HT neurons. Consequently, the possibility of affecting neurons other than serotonergic ones when performing neurotoxic lesions is a crucial variable that has to be considered. So, the effects of 5, 7-DHT lesion on the level of NA, DA, HVA and DOPAC were determined by the HPLC. 5, 7-DHT had no effect on the level of NA, DA, HVA and DOPAC. These results are in harmony with previous reports^{294, 297}.

Since 5, 7-DHT is structurally very similar to 5-HT, and antagonizes 5-HT uptake by synaptosomes and brain slices, the presumption was that the selectivity of this drug in destroying 5-HT terminals was related to its rapid concentration in 5-HT cellular elements, mediated via the 5-HT reuptake carrier. The actual destructive mechanism was viewed as non-selective²⁹⁸. If the specificity of 5, 7-DHT as a 5-HT neurotoxin depends on its concentration in the 5-HT neuron via the 5-HT transporter, it should be possible to block 5, 7-DHT induced 5-HT depletion by pretreating animals with a SSRIs, such as fluoxetine or chlorimipramine. But curiously, on the few occasions when this has been attempted, SSRIs failed to prevent 5-HT depletion by 5, 7-DHT²⁵⁶. In our study, tramadol, which is known to inhibit 5-HT reuptake, also did not manage to change the effects of 5, 7-DHT. On the other hand, this inability of the 5-HT reuptake blockers to prevent 5-HT depletion appears not to be explained by a high concentration of the neurotoxin relative to that of the reuptake blocker, since Choi et al.²⁵⁶ employed higher doses of reuptake blockers and showed that low doses of 5, 7-DHT also depleted 5-HT in that study. The mechanism by which 5, 7-DHT produces its depletion of neuronal 5-HT stores in brain thus remains unknown.

On the other hand, the UCMS regimen decreased 5-HT, 5-HIAA, NA and DA but not DOPAC and HVA level in some brain regions when compared to non-stressed mice. Indeed, many studies have reported abnormalities in the biogenic amines and their metabolites in blood, urine and CSF in depressed patients²⁹⁹. For example, the 5-HT content in brains of suicide victims was found to be low as compared with controls. In addition, there was some evidence that there was decrease in the 5-HIAA level, in the depressed patients³⁰⁰.

The chronic treatment with tramadol increased the level of 5-HT in the frontal cortex, the hippocampus and the region of the raphe magnus whereas it augmented the level of 5-HIAA in the striatum and the region of the raphe magnus in sham stressed

mice. The effects of chronic tramadol in the present study are consistent with previous studies demonstrating an increase in extracellular 5-HT level in different brain areas following chronic administration with different SSRIs^{301, 302}. Indeed, the effects of tramadol on the 5-HT level in the hippocampus, which has a rich serotonergic innervation²⁵⁵, are similar to most of the antidepressants such as citalopram³⁰³. It is well known that tramadol shows its effect enhancing the extraneuronal concentration of NA and 5-HT by interfering with both the reuptake and release mechanisms^{4, 304}. Tramadol not only blocks the reuptake of 5-HT in the DRN, it also enhances the 5-HT level³⁰⁴. Interestingly, the effects of tramadol on 5-HT efflux and reuptake were of a similar magnitude whereas the effect of paroxetine on the 5-HT reuptake is much greater than that on 5-HT efflux because of negative feedback mechanisms reducing the efflux³⁰⁵.

Moreover, tramadol enhanced the level of NA in the striatum, the hippocampus and the region of the raphe magnus. The relation among the noradrenergic system, depression and the limbic system is well known, but there are few studies about the effects of tramadol on the level of NA in brain regions. For example, Halfpenny et al.³⁰⁶ reported that tramadol induces a NA efflux in the LC. So, it's possible that the augmentation of the NA level in the striatum, the hippocampus and the region of the raphe magnus is related to the innervation of LC. Indeed, there are projections from the LC to these brain areas. To clarify the effects of tramadol on the NA level in the region of the LC, we determined the NA level in this region and this will discuss in the following pages.

However, the 5, 7-DHT lesion antagonized the effects of desipramine on the state of the coat of mice and in the splash test, desipramine did not manage to change the level of 5-HT and 5-HIAA level in lesioned and sham stressed mice except in the hippocampus. In this region it significantly augmented the level of 5-HT. In addition, 5,7-DHT did not change NA level. So, the antagonism of effect of desipramine by the neurotoxin cannot be related with the noradrenergic system.

On the other hand, desipramine augmented the level of NA in the striatum, the hippocampus and the region of the raphe magnus. It has already been reported that desipramine increased the level of NA in the striatum whereas it decreased the NA level in hippocampus after 3 weeks of exposure³⁰⁷. It is possible that the increased concentration of hippocampal NA observed in the present study resulted from the

decreased sensitivity of α_2 -adrenoreceptors, which inhibit NA release. Indeed, the chronic treatment of TCA down-regulate, α_2 -adrenoreceptors³⁰⁸.

Furthermore, the chronic treatment with desipramine and tramadol increased the DA level in the striatum but not other brain regions. Different from the our results Tanda et al.³⁰⁹ reported that chronic desipramine increased extracellular NA and DA by three-fold as compared to saline controls in the prefrontal cortex. These authors suggested that prefrontal cortex DA plays an important role in the antidepressant properties of desipramine. Several studies have demonstrated that tramadol enhances DA release in specific brain areas including medulla oblongata, hypothalamus, corpus striatum and nucleus accumbens^{231, 310}. In addition, autoradiographic analysis of [³H] 7 OH-DPAT and [³H] raclopride (D₂-dopamine receptor ligand) binding revealed a significant up-regulation of D₂ and D₃ receptors in the rat nucleus accumbens upon repeated treatment with tramadol³¹¹.

On the other hand, tramadol and desipramine can enhance DA level by their effects on the noradrenergic system. Indeed, within mesocorticolimbic DA system, several interactions between DA and NA neurotransmission have been clearly described³¹².

In conclusion, the administration of 5, 7-DHT into the DRN decreased the 5-HT and 5-HIAA level in some brain areas without changing the NA, DA, HVA and DOPAC level. Chronic treatment with tramadol and desipramine showed antidepressant-like effects in stressed sham but not in stressed lesioned groups. While the level of 5-HT, 5-HIAA, NA and DA diminished by UCMS regimen in some brain areas, tramadol and/or desipramine augmented the level of these monoamines in certain brain regions. Thus, it is possible to suggest that the serotonergic system has an important role in the antidepressant-like effects of tramadol and desipramine. And it is worthwhile to clarify the receptors which can mediate the action of these drugs.

5.3.2. The Effects of Pindolol on the Antidepressant-like Effects of Tramadol and Desipramine in the UCMS Model in BALB/c Mice

5-HT_{1A} receptors are intimately involved in the mechanism of action of antidepressant drugs and also according to the results that we obtained from the previous part, we decided to search the effects of 5-HT_{1A} receptors on the antidepressant-like effects of tramadol and desipramine. We examined the effects of 5-HT_{1A} receptor ligands on the antidepressant-like effects of tramadol and desipramine by using pindolol. Pindolol is a 5-HT_{1A/1B} receptor antagonist and a weak β -adrenoreceptor blocker. When given alone at the concentrations used in this work, pindolol did not change the state of the coat and grooming behaviour in the splash test when compared to vehicle group. Furthermore, during the UCMS regimen, we observed neither a significant acceleration nor diminution of the onset of the antidepressant-like actions of desipramine and tramadol by pindolol.

Most antidepressant drugs, such as SSRI, elicit their effects via an increase of the 5-HT level by preventing its reuptake. However, this increase is offset by a negative feedback because of the activation of 5-HT_{1A} autoreceptors³¹³. It has already been reported that 5-HT_{1A} receptor antagonists such as pindolol could accelerate the clinical effects of antidepressants like SSRI by preventing this negative feedback³¹⁴. It was also reported that the combined treatment with fluoxetine and pindolol produced higher response rate and faster onset for a higher sustained response than fluoxetine alone³¹⁵. Furthermore, another double blind study showed that the combined treatment with paroxetine and pindolol for 4 weeks induced a significantly greater response rate than treatment with paroxetine alone³¹⁶. This effect of pindolol is attributed to its affinity to 5-HT_{1A} receptors, as another β adrenergic receptor blocker (metoprolol) that has no significant affinity for 5-HT_{1A} receptors produced no acceleration or potentiation of the antidepressant effect of paroxetine³¹⁶.

According to our results that tramadol increases the level of 5-HT and Bamigbade et al.³⁰⁴'s that showed the inhibition of the re-uptake of 5-HT in the raphe nuclei by the tramadol exposure, suggest a possible implication of these nuclei in the effects of tramadol. Considering that tramadol acts like a SSRIs, it could be hypothesized that the combination with pindolol can block the negative feedback at the raphe level and potentiate the effect of tramadol. Alternatively, pindolol can possess

antagonistic properties at the level of post-synaptic 5-HT_{1A} receptors in forebrain, and reduce the effects of tramadol. In our study, we did not observe any effects of pindolol. Thus, it is possible that the blockade of forebrain 5-HT_{1A} receptor may mask the increase of 5-HT release. In addition, two studies of pindolol combined with fluoxetine³¹⁷ found no acceleration of the antidepressant response. In contrast, the antinociceptive effect of tramadol was enhanced by co-administration of both pindolol and WAY 100635 (5-HT_{1A} receptor antagonists), and reduced by the selective agonist of 5-HT_{1A} receptors, 8-OH-DPAT^{318, 319} whereas the antidepressant-like effects of tramadol was blocked by WAY 100635 in the FST¹⁰. However, Frink et al.²³¹ reported that tramadol and its main metabolite have no affinity to 5-HT_{1A} receptors

The results that we obtained from the study that examined the effects of 5, 7 DHT lesion on the antidepressant-like effects of desipramine gave us an idea whether desipramine can have an effect on serotonergic receptors, especially on the 5-HT_{1A} receptor. So, we also tested the effects of pindolol on the antidepressant effect of desipramine. In our experiment setting, pindolol did not change the antidepressant-like effects of desipramine. It has been shown that the combination of the tricyclic antidepressant drugs lacking effect on 5-HT reuptake (desipramine or trimipramine) with pindolol resulted in only one of ten patients achieving a 50% improvement after 28 days. In contrast, the combination of the SSRI fluvoxamine with pindolol produced a marked antidepressant effect¹⁴. Moreover, in the FST, pre-treatment with (+/-) pindolol potentiated the effects of SSRI and was devoid of any activity on desipramine²⁰². In addition, the studies made with 5-HT_{1A} knockout mice showed that the function of 5-HT_{1A} receptors (likely postsynaptic) is necessary for the expression of fluoxetine's behavioural effects, whereas they were unnecessary for the antidepressant-like effects of desipramine in TST²⁰¹. These results provide further evidence that pindolol does not accelerate the antidepressant effect of drugs that alter the noradrenergic function.

Although pindolol can block the 5-HT_{1A} autoreceptor, it is inactive at post-synaptic 5-HT_{1A} receptors³²⁰. It was also suggested that the antidepressant-like effects of desipramine are seem to be mediated by post-synaptic 5-HT_{1A} receptors²⁰².

Altogether, it is possible to suggest that the antidepressant-like effect of tramadol and desipramine is not mediated by the presynaptic 5-HT_{1A} receptors.

5.4. The Participation of the Noradrenergic System in the Antidepressant-like Effects of Tramadol and Desipramine in the UCMS Model in BALB/c Mice

In this part of the study, we aimed to clarify the role of the noradrenergic system in the antidepressant-like effects of desipramine and tramadol in the UCMS model in BALB/c mice.

5.4.1. The Effects of Tramadol and Desipramine Treatment on the NA and of its Metabolite MHPG Level

In this part we sought the effects of tramadol and desipramine on the NA level and of its metabolite MHPG in stressed mice. Substantial evidence suggests that MHPG and NA levels could be possible indices of central noradrenergic function. Measurement of MHPG level in body fluids has been most widely applied to studies of depression and in attempts to predict the likelihood of a therapeutic response to antidepressant drugs. It should be noticed that about one-third of plasma MHPG is of brain origin. For that reason, we measured the MHPG level directly in brain regions by HPLC.

The results of HPLC presented herein indicate that the levels of NA and/or MHPG were augmented by chronic desipramine and/or tramadol in several brain areas, including LC, hypothalamus and hippocampus. No effect was observed in the cerebellum.

We showed that chronic desipramine treatment induced a large increase in NA and MHPG in the LC, which confirms data from others. Indeed, it has already been shown that chronic treatment with desipramine elevates NA transporter mRNA³²¹ and increases extracellular NA concentration²⁴⁷ in the LC in non-stressed animals. The increase in the NA and MHPG level is also obtained after chronic tramadol treatment, which may be explained by the ability of this compound to act as a NARI in this area³⁰⁶. However, it should be noticed here that other studies did not always find similar results. For example, Hopwood et al.²³⁹ have examined the effects of chronic tramadol on NA efflux and uptake in LC using long duration electrical stimulations in naive rats. They showed that chronic tramadol had no effect on NA uptake and NA efflux in the LC. This discrepancy may be related to the fact that we used stressed mice instead of naive mice. Indeed, we have already shown that tramadol shows its antidepressant-like

effects in stressed mice, but not in non-stressed mice. So it is possible to suggest that tramadol shows its effect on the level of NA only in stressed mice. Indeed, in the previous part measuring the NA level in the lesioned and sham mice also showed that tramadol significantly augmented NA level in stressed mice but not non-stressed mice.

There are number of projections radiating from LC to regions of the brain including the thalamus, the cortex, the amygdala, the hippocampus and the hypothalamus which are key areas to understand the anatomical basis of major psychiatric disease, particularly stress-induced illness and depression^{15, 16}. So, we also focused our interest on two distinct brain regions; the hypothalamus and the hippocampus that are involved in emotion, motivation, learning and memory functions, which are related to some symptoms of depression³².

We showed that chronic desipramine and tramadol exposure significantly increased the level of MHPG in the hypothalamus while the augmentation of NA level in this region was slight. On the other hand, in the hippocampus, the level of MHPG and NA were augmented significantly by tramadol while desipramine only increased significantly the level of NA without changing the MHPG level. Wu et al.³²² showed opposite results as chronic administration of the selective NARI MCI-225 increased extracellular NA level in non-stressed rats but not in stressed-rat in hypothalamus. In addition, Chung et al.³⁰⁷ showed that the concentration of NA was decreased in the hippocampus and in the thalamus after 3 weeks of desipramine treatment. However, the present study and these previous reports differ fundamentally e.g, in terms of type of stress, species used, the duration of treatments.

According to our results it is possible to suggest that chronic administration of desipramine and tramadol increases the NA and MHPG levels in stressed mice, albeit in a brain region specific manner. We then thought that it is necessary to clarify the type of adrenoceptors, which can have a role in the alteration of NA and MHPG levels by these drugs.

5.4.2. The Effects of Propranolol, ICI 118,551 and Yohimbine on the Antidepressant-like Effects of Tramadol and Desipramine in the UCMS Model in BALB/c Mice

We also investigated the effects of β and α_2 adrenoreceptors on the antidepressant-like effects of tramadol and desipramine. We showed that the non-selective β -adrenoceptor antagonist propranolol significantly antagonized the effects of desipramine and tramadol in the UCMS model, while it did not elicit any intrinsic effect. It may be possible that propranolol diminishes the antidepressant-like effects of tramadol and desipramine by reversing their action on the β -adrenoceptors. Indeed, it is well known that chronic treatment of animals with several antidepressants produces a significant reduction in the number of β -adrenoceptors in the brain³²³. In addition, Hopwood et al.²³⁹ reported that chronic tramadol exposure significantly decreased frontocortical β -adrenoceptors as seen with most classical antidepressants. Likewise, repeated administration of desipramine caused the expected decrease in the Bmax value of β -adrenoceptors³²⁴.

On the other hand, Asakura et al.³²⁴ showed that the β -adrenoceptor down-regulation is induced by acute and chronic exposure to moderate and predictable stress, while chronic unpredictable stress up-regulated these receptors. So, it is possible to suggest that tramadol and desipramine counteract the effects of UCMS on the β -adrenoceptors.

We also used selective β_2 -adrenoceptor antagonist ICI 118,551 to clarify the implication of β -adrenoceptors. ICI 118,551 antagonized the antidepressant-like effects of desipramine and tramadol on the state of the coat and in the splash test in stressed mice at a dose that selectively blocks β_2 -receptors. Murugaiah and O'Donnell.³²⁵ demonstrated that the β -adrenergic agonist-induced facilitation of stimulation-evoked [³H]-NA release from rat cerebral cortical slices is primarily due to activation β_2 -adrenergic receptors. Decreases in β_2 -adrenoceptor density after chronic treatment with antidepressant have already been reported³²⁶. In contrast, Nelson et al.³²⁷ showed that chronic desipramine exposure caused a significant reduction in the density of β_1 but not β_2 -adrenoceptors. However, according to our results, desipramine may use β_2 -adrenoceptors to show its antidepressant-like effects. On the other hand, brain areas in

which we observed augmentation of NA level by tramadol and desipramine are the region rich of β_2 -adrenoceptors³²⁸. So, it is possible that the β -adrenoceptors, particularly the β_2 - subtype, have an important participation in the antidepressant-like effects of tramadol and desipramine.

We also searched the effects of α_2 receptor antagonist yohimbine on the antidepressant-like effects of tramadol and desipramine. It is well known that yohimbine is highly selective for α_2 over α_1 adrenergic receptors and is frequently used to assess the involvement of α_2 -adrenergic receptors in the mechanism of action of drugs³²⁹. Yohimbine significantly diminished the antidepressant-like actions of both desipramine and tramadol over coat state and on total frequency of the grooming behaviour during the splash test. In accordance with our results, the reduction in immobility induced by tramadol in the FST was reversed by both non-specific α antagonist phentolamine and by the α_2 -adrenergic receptor antagonist yohimbine⁶. In addition, it has been recently shown that repeated treatment with tramadol (20 mg/kg, i.p, once daily for 21 days) induces downregulation of [³H]RX821002 binding sites, a selective α_2 -adrenergic receptor ligand, in the rat brain²⁴⁰. Subhash et al.³³⁰ reported that the density of rat cortical α_2 -adrenergic receptors was significantly decreased upon repeated treatment with tricyclic antidepressants. Also, Giralt and Garcia-Sevilla.³³¹ showed that chronic but not short term treatment with drugs, which antagonize endogenous NA like yohimbine, upregulated α adrenoceptors. Moreover, after the chronic administration of idazoxan (α_2 -adrenoceptor antagonist), α_2 -adrenoceptor number has been reported to be significantly increased and plasma MHPG levels has been reported to be significantly reduced by chronic idazoxan³³². According to our results, it can be suggested that yohimbine may diminish the antidepressant-like effects of tramadol by reversing its down-regulative action on α_2 -adrenergic receptors.

Besides, it has been shown that the effect of desipramine on extracellular NA in the brain cortex is modulated by α_2 -adrenoceptors in the LC²⁴⁷. In addition, Reneric et al.³³³ reported that idazoxan produced no anti-immobility effects *per se* in the FST and antagonized the effects of desipramine.

In the light of these results, it is possible that the noradrenergic system especially β and α_2 adrenoreceptors contribute to the antidepressant-like effects of tramadol and desipramine.

5.5. GENERAL DISCUSSION

In the present study we searched the possible antidepressant-like effects of tramadol in the UCMS. When we started to realize this thesis, there were few papers about possible antidepressant-like effects of tramadol. These studies had mentioned the necessity of other researchs about the antidepressant-like effects of tramadol. So, we sought the antidepressant-like effects of tramadol in the UCMS model and we determined the possible mechanisms underlying its action.

The results obtained from this thesis show that tramadol has an antidepressant-like effect, which is similar to available antidepressants, in the UCMS in BALB/c mice. To search the mechanisms underlying the antidepressant-like effects of tramadol, two systems, which implicate the pathophysiology of major depression and the mechanism of analgesic effect of tramadol were chosen; the serotonergic and noradrenergic system. However, the brain function may not be investigated systemically since most studies dealt with the whole brain or only a couple of structures of the brain with specific amines. Therefore, the present study aimed to investigate different effects of 5, 7-DHT neurotoxin, UCMS and tramadol or desipramine treatment on the different brain regions. Interestingly, our results may answer the frequently asked question whether the low level of 5-HT induces depression or depression decreases the level of 5-HT. Indeed, 5, 7-DHT lesioned non-stressed mice did not show any symptoms of depression such as decreased grooming or dirty coat state whereas they have a low 5-HT level in the hippocampus, the striatum and the frontal cortex. The decrement of 5-HT level by the administration of 5, 7-DHT into the DRN blocked the antidepressant-like effects of desipramine and tramadol. In addition tramadol increased the 5-HT, DA and NA level whereas desipramine increased NA and DA level in the sham stressed mice. These effects were observed in the brain regions which are important areas for mood disorders such as the hippocampus, the frontal cortex and the striatum. Desipramine also augmented the level of 5-HT in the hippocampus in lesioned stressed mice. All these results suggest that tramadol and desipramine altered neurotransmission according to the brain region and that NA, 5-HT and DA may have an important role in the expression of antidepressant action of these drugs. According to the results that we obtained from this part of the study we were interested in the participation of 5-HT_{1A} receptors in the antidepressant-like effects of tramadol and desipramine. Indeed, among

the numerous 5-HT receptors, the 5-HT_{1A} receptors seem to be particularly remarkable targets as to the action of antidepressants. Santarelli et al.³³ reported that 5-HT_{1A} receptors are required for fluoxetine induced neurogenesis, a process necessary to its antidepressant effects. Furthermore, several studies have demonstrated that combination of a 5-HT_{1A} receptor antagonist with SSRIs potentiates the effect of antidepressant drug on 5-HT release^{14, 334}. There is also some evidence showing the interaction between desipramine and 5-HT_{1A} receptors²⁰². In this study pindolol was not capable to change the antidepressant-like effects of tramadol or desipramine at the dose used. Although pindolol can block the 5-HT_{1A} autoreceptor, it is inactive at post-synaptic 5-HT_{1A} receptors³²⁰. So it may be possible that tramadol and desipramine act on post-synaptic 5-HT_{1A} receptors. Indeed, it was also suggested that antidepressant-like effects of desipramine seem to be mediated by post-synaptic 5-HT_{1A} receptors²⁰². Moreover, Berrocoso et al.¹⁰ reported that 5-HT_{1A} receptors located in the forebrain are responsible for the antidepressant-like effects of tramadol.

On the other hand, the chronic treatment with tramadol and/or desipramine augmented NA and/or MHPG level in some brain regions in stressed mice. Moreover, the effects of these drugs were antagonized by the chronic treatment of propranolol, ICI 118,551 and yohimbine.

The augmentation of the NA and MHPG level in the certain brain regions especially limbic areas, but not in the cerebellum, by tramadol and desipramine treatment can occur via the activation of β -adrenoceptors induced by these drugs. It has been reported that stimulation of β -adrenoceptors by nonselective β -adrenoceptor agonist isoproterenol enhance NA release from rat cerebral cortical, hypothalamic and hippocampal slices; this release mechanism appears to involve both β_1 and β_2 subtype³³⁵. Also in brain samples obtained postmortem from antidepressant-free suicides with a retrospective diagnosis of depression, the number of α_2 -adrenoceptors was significantly higher in frontal and temporal cortices or the hippocampus, as well as in the LC³³⁶⁻³³⁸. As a result, we have determined the effects of yohimbine administration on the antidepressant-like effects of tramadol and we showed that yohimbine antagonized the effects of tramadol. In connection with the serotonin hypothesis, α_2 -adrenergic receptor down-regulation has been revitalized²⁴⁰, since the down-regulation of α_2 -adrenergic heteroreceptors controlling 5-HT release may be an important factor in

antidepressant action. Hence, the robust downregulation of these receptors induced by tramadol, as discussed previously, could result in a significant increase in the amount of 5-HT in synaptic cleft.

The present results demonstrate that serotonergic and noradrenergic systems are indispensable for the antidepressant-like action of tramadol. Indeed, when we blocked either serotonergic system or noradrenergic system via lesion or receptor antagonists, the antidepressant-like effects of tramadol were diminished. Thus, if one of these systems doesn't function normally, the other one doesn't compensate it.

6. CONCLUSIONS and PERSPECTIVE

By interpreting the data of our study, we suggest that UCMS significantly induced physical and behavioural abnormalities such as a degradation of the coat state and decreased the grooming behaviour in BALB/c mice, which were reversed by a chronic administration of desipramine and tramadol.

In the light of the results, it is possible to suggest that the serotonergic and noradrenergic system contribute to the antidepressant-like effects of tramadol and desipramine. Needless to say, further studies should be undertaken to explore the role of other neurotransmitters in the antidepressant-like action of tramadol. Indeed, the results that we obtained from the HPLC show that tramadol and desipramine augment the level of DA in the striatum. Therefore, it is possible that the dopaminergic system has also a role in the antidepressant-like effects of these drugs. The effects of tramadol on the HPA axis or neuropeptides can be also investigated. Besides, in one of our previous study, we showed that the nitrenergic system contributes to the antinociceptive effects of tramadol³³⁹. Thus, it can be possible that the nitrenergic system can also participate the antidepressant-like effect of tramadol. Moreover, additional experiments, using specific 5-HT_{1A} agonist and antagonists with different doses are necessary to suggest more reasonable explanations for the lack of action of pindolol. This work can also be enhanced by the inclusion of autoradiographic or in situ hybridisation studies of serotonergic and noradrenergic receptors.

On the other hand, further clinical studies are needed to explore the efficacy of antidepressant activity since the neurochemical data and the results obtained from animal studies of depression so far indicate such a potential.

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